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**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Code assigned:** | ***2017.009D*** | | | | (to be completed by ICTV officers) |
| **Short title:** Two new species in each of the genera *Circovirus* and *Cyclovirus* | | | | | |
| **Modules attached**  (Modules 1, 4 and either 2 or 3 are required. | | **1**  **2  3  4** | | | |
| **Author(s):** | | | | | |
| Arvind Varsani, Karyna Rosario, Joaquim Segalés, Eric Delwart, Mya Breitbart | | | | | |
| **Corresponding author with e-mail address:** | | | | | |
| Arvind.varsani@asu.edu | | | | | |
| **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) | | | **Circoviridae Study Group** | | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | | | |
|  | | | | | |
|  | | | | | |
| Date first submitted to ICTV: | | | | 5 June 2017 | |
| Date of this revision (if different to above): | | | | 20 June 2017 | |

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| **ICTV-EC comments and response of the proposer:** |
|  |

**Part 2**: **PROPOSED TAXONOMY**

|  |
| --- |
| Present the proposed new taxonomy on accompanying spreadsheet |
| **Name of accompanying spreadsheet:** 2017.009D.N.v1.Circoviridae\_4sp |

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:** |
| Ku, X., Chen, F., Li, P., Wang, Y., Yu, X., Fan, S., Qian, P., Wu, M., He, Q., 2017. Identification and genetic characterization of porcine circovirus type 3 in China. Transbound Emerg Dis 64(3), 703-708.  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(9), e108277.  Palinski, R., Pineyro, P., Shang, P.C., Yuan, F.F., Guo, R., Fang, Y., Byers, E., Hause, B.M., 2017. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. J Virol 91(1), pii: e01879-16.  Phan, T.G., Giannitti, F., Rossow, S., Marthaler, D., Knutson, T.P., Li, L., Deng, X., Resende, T., Vannucci, F., Delwart, E., 2016. Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. Virol J 13(1), 184.  Rosario, K., Breitbart, M., Harrach, B., Segales, J., Delwart, E., Biagini, P., Varsani, A., 2017. Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. Arch Virol 162(5), 1447-1463.  Shen, H., Liu, X., Zhang, P., Wang, L., Liu, Y., Zhang, L., Liang, P., Song, C., 2017. Genome characterization of a porcine circovirus type 3 in South China. Transbound Emerg Dis. doi: 10.1111/tbed.12639. [Epub ahead of print] |

|  |
| --- |
| **Annex:**  Please explain the reasons for the taxonomic changes you are proposing and provide evidence to support them. The following information should be provided, where relevant:   * **Species demarcation criteria**: Explain how new species differ from others in the genus and demonstrate that these differences meet the criteria previously established for demarcating between species. If no criteriahave previously been established, and if there will now be more than one species in the genus, please state the demarcation criteria you are proposing. * **Higher taxa**:   + There is no formal requirement to state demarcation criteria when proposing new genera or other higher taxa. However, a similar concept should apply in pursuit of a rational and consistent virus taxonomy.   + Please indicate the **origin of names** assigned to new taxa at genus level and above.   + For each new genus a **type species** must be designated to represent it. Please explain your choice. * **Supporting evidence**: The use of Figures and Tables is strongly recommended (note that copying from publications will require permission from the copyright holder). For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance. |

The family *Circoviridae* has two genera, *Circovirus* and *Cyclovirus* (Rosario et al., 2017). All members of the family *Circoviridae* have circular single-stranded DNA genomes with ambisense genome organization. In the case of circoviruses, the replication-associated protein (Rep) open reading frame (ORF) is located on the putative virion sense strand whereas the capsid protein (CP) is on the complementary strand. In the case of the cycloviruses, the genome organization is mirror opposite to that of circoviruses (Rosario et al., 2017).

Rosario et al. (2017) provided a recent update on the taxonomy of the family *Circoviridae*. Based on the comprehensive analysis of the distribution of pairwise identities (PI) among cyclovirus and circovirus genomes, a 80% PI threshold was determined to be best for species classification within the family *Circoviridae* (Rosario et al., 2017). Using the 80% PI threshold calculated using SDT v1.2, Muhire et al. (2014), coupled with phylogenetic support, 27 circovirus species and 43 cyclovirus species are currently recognized by ICTV.

***Circovirus***

Two new circovirus species have recently been identified from bats (n=3) and pigs (n=15). All of the isolates identified for each of these novel species contain a genome organization that is characteristic of circovirus species. The 18 isolates (Table 1), based on PI analysis (Figure 1) coupled with phylogenetic support (Figure 2), are members of two new species

1. porcine circovirus 3 (n=15)
2. bat associated circovirus 9 (n=3)

The fifteen porcine circovirus 3 isolates have been identified in pigs from USA (n=3) and China (n=12) (Ku et al., 2017; Palinski et al., 2017; Phan et al., 2016; Shen et al., 2017). These isolates share >98% PI. These isolate genome sequences share <80% PI with all other circovirus sequences.

The three bat associated circovirus 9 isolates share >80% PI among each other. These isolate sequences share <80% PIs with all other circovirus sequences (Figure 2).

***Cyclovirus***

Two new cyclovirus isolates have been recently identified from human cerebrospinal fluid and a mouse spleen. These two sequences are available in GenBank (accession no. KT878836 and KU053483) but, currently, there are no peer-reviewed publications associated with these genomes. Both of the new cyclovirus isolates contain a genome organization characteristic of cyclovirus species.

The two isolates (Table 1), based on PI analysis (Figure 3) coupled with phylogenetic support (Figure 4), are members of two new species

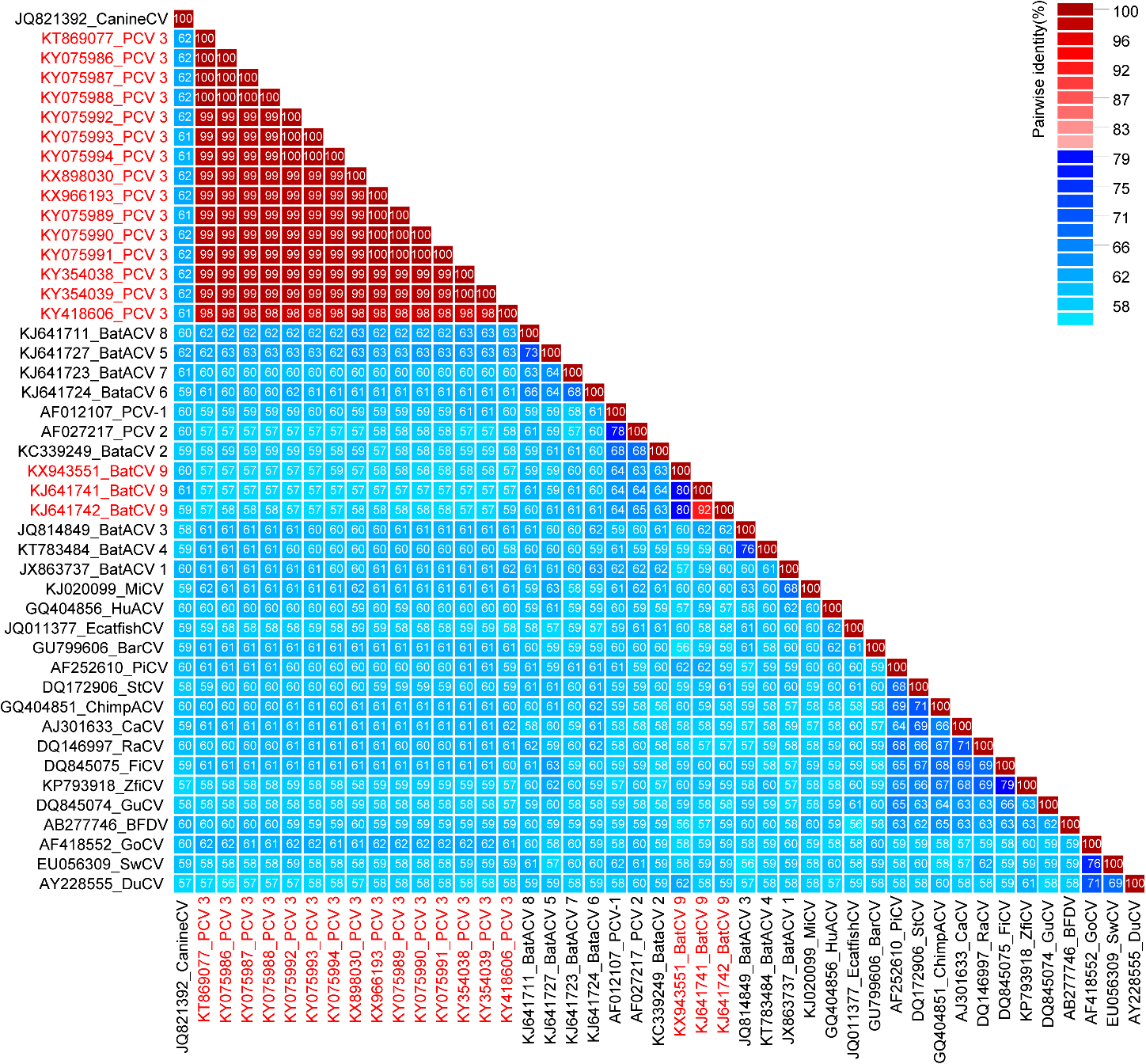
1. Human associated cyclovirus 12 (n=1)
2. Mouse associated cyclovirus 1 (n=1)

***Genera confirmation using Rep-based analysis***

The Reps of the new isolates together with those representative sequences from each circovirus and cyclovirus species were aligned and the alignment was used to infer a Maximum likelihood phylogenetic tree (Figure 5). The Rep-based phylogenetic tree support the classification of porcine circovirus 3 and bat associated circovirus 9 to the genus *Circovirus* and human associated cyclovirus 12 and mouse associated cyclovirus 1 to the genus *Cyclovirus*.

**Table 1:** Summary of new circoviruses and cycloviruses

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Genus** | **Virus** | **GenBank accession #** | **Acronym** | **Isolate** | **Source** | **Country** |
| *Circovirus* | porcine circovirus 3 | KT869077 | PCV-3 | 29160 | pig | USA |
|  |  | KX898030 | PCV-3 | PCV3-US/MN2016 | pig | USA |
|  |  | KX966193 | PCV-3 | PCV3-US/SD2016 | pig | USA |
|  |  | KY075986 | PCV-3 | PCV3/CN/Fujian-5/2016 | pig | China |
|  |  | KY075987 | PCV-3 | PCV3/CN/Fujian-12/2016 | pig | China |
|  |  | KY075988 | PCV-3 | PCV3/CN/Henan-13/2016 | pig | China |
|  |  | KY075989 | PCV-3 | PCV3/CN/Jiangxi-62/2016 | pig | China |
|  |  | KY075990 | PCV-3 | PCV3/CN/Chongqing-147/2016 | pig | China |
|  |  | KY075991 | PCV-3 | PCV3/CN/Chongqing-148/2016 | pig | China |
|  |  | KY075992 | PCV-3 | PCV3/CN/Chongqing-150/2016 | pig | China |
|  |  | KY075993 | PCV-3 | PCV3/CN/Chongqing-155/2016 | pig | China |
|  |  | KY075994 | PCV-3 | PCV3/CN/Chongqing-156/2016 | pig | China |
|  |  | KY354038 | PCV-3 | CN/Hubei-610/2016 | pig | China |
|  |  | KY354039 | PCV-3 | CN/Hubei-618/2016 | pig | China |
|  |  | KY418606 | PCV-3 | PCV3-China/GD2016 | pig | China |
|  | bat associated circovirus 9 | KX943551 | BatACV-9 | DaiShan | *Myotis davidii* | China |
|  |  | KJ641741 | BatACV-9 | BtRf-CV-61/YN2010 | *Rhinolophus ferrumequinum* | China |
|  |  | KJ641742 | BatACV-9 | BtRf-CV-62/YN2010 | *Rhinolophus ferrumequinum* | China |
| *Cyclovirus* | mouse associated cyclovirus 1 | KT878836 | MoACyV-1 | Cyclo-sf1 | mouse spleen | USA |
|  | human associated cyclovirus 12 | KU053483 | HuACyV-12 | IECSF08 | human cerebrospinal fluid | India |



**Figure 1:** Genome-wide pairwise identities among circoviruses determined using SDT v1.2 (Muhire et al., 2014) with a ‘two color’ profile highlighting. The 18 new circovirus isolates (highlighted in red font) represent two new species based on the 80% PI threshold.

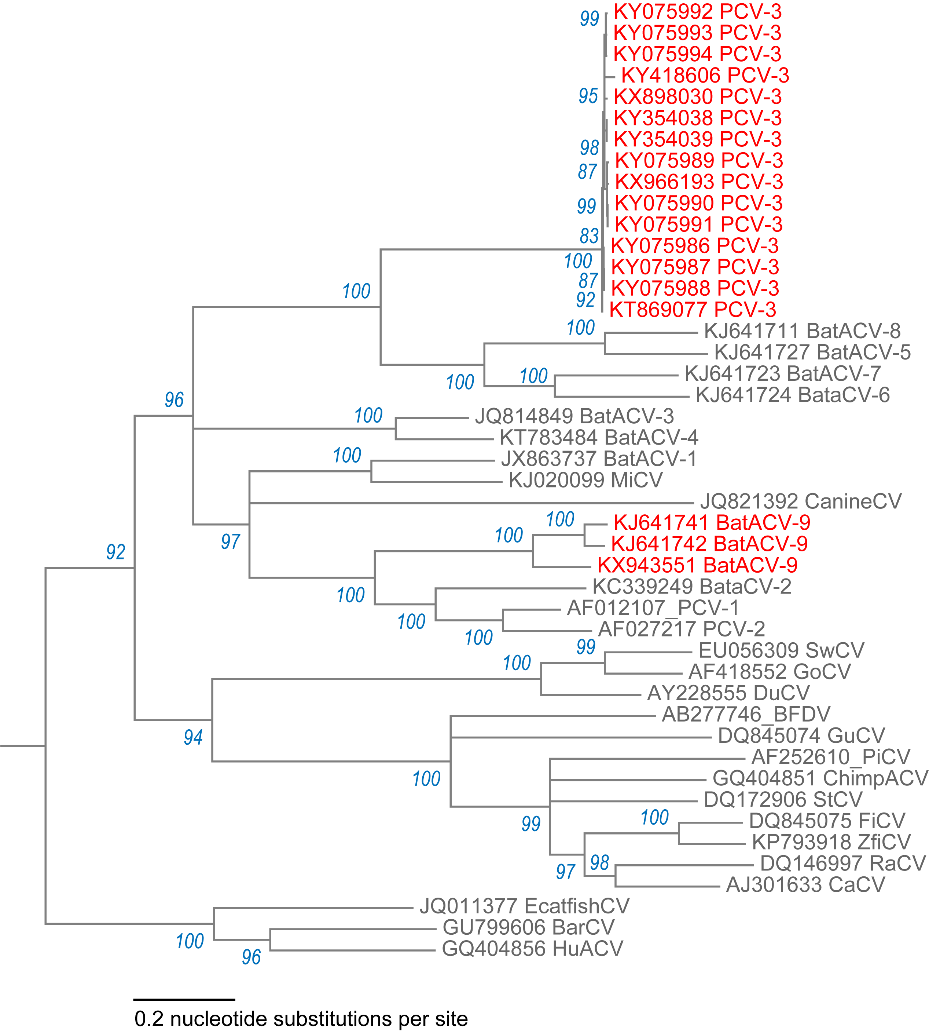
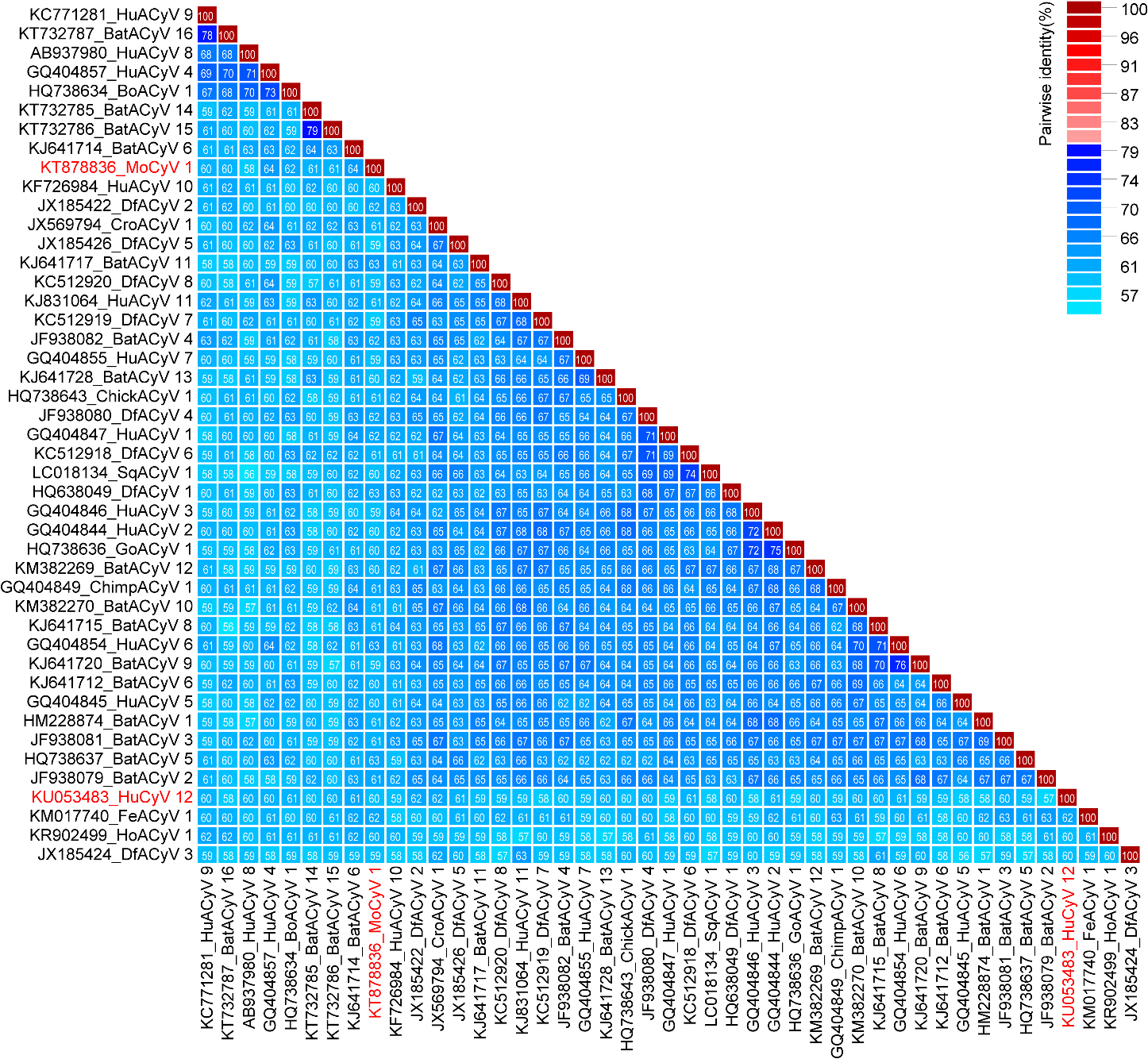
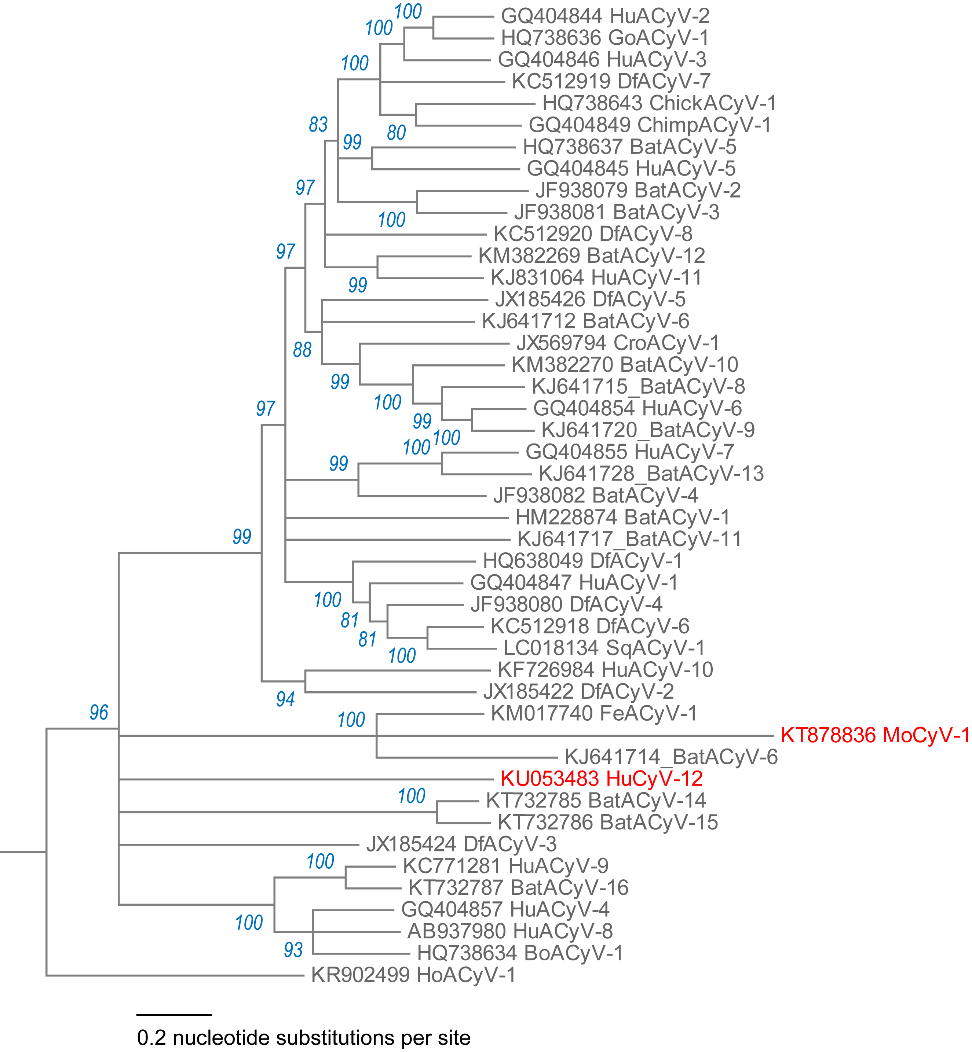


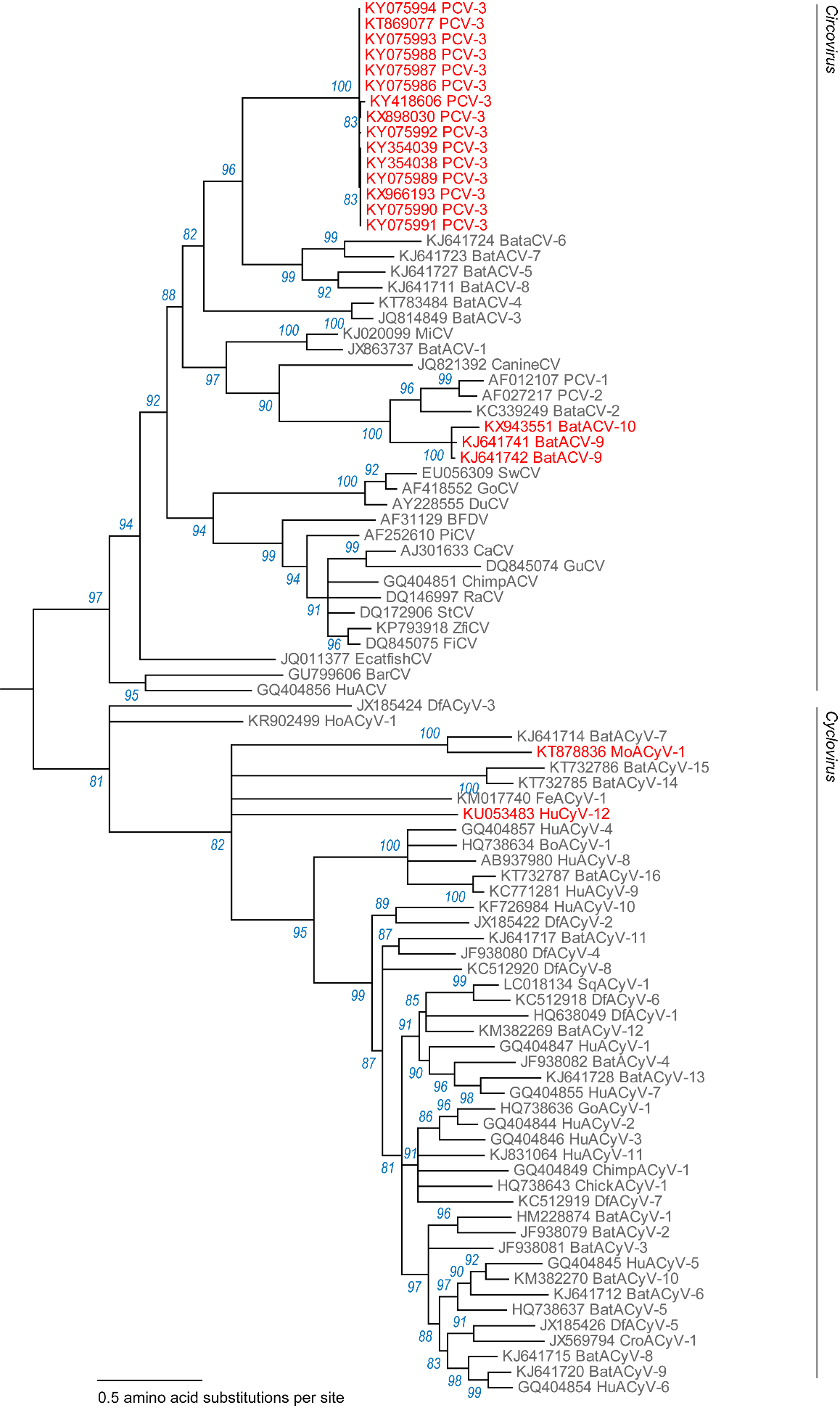
Figure 2: Maximum likelihood phylogenetic tree of representative circovirus species. The ML tree was constructed after aligning complete genome sequences and inferred using PHYML with GTR+G model of substitution (with SH-like branch support). Branches with <80% support have been collapsed. The phylogenetic tree was rooted after using cyclovirus reverse complemented genome sequences as an outgroup. Members of the two new species are highlighted in red font.



**Figure 3:** Genome-wide pairwise identities among cycloviruses determined using SDT v1.2 (Muhire et al., 2014) with a ‘two colour’ profile highlighting. The two new cyclovirus species are highlighted with red font.



**Figure 4:** Maximum likelihood phylogenetic tree of representative cyclovirus species. The ML tree was constructed after aligning complete genome sequences and inferred using PHYML with GTR+G model of substitution (with SH-like branch support). Branches with <80% support have been collapsed. The phylogenetic tree was rooted after using circovirus reverse complemented genome sequences as an outgroup. The two new cyclovirus species are highlighted in red font.



**Figure 5:** Maximum likelihood phylogenetic tree of representative replication-associated protein (Rep) amino acid sequences. The ML tree showing the relationship between cyclovirus and circovirus Reps was inferred using PHYML with LG+G model of substitution (with SH-like branch support) and rooted with Rep sequences from members of the family *Genomoviridae*. Branches with <80% support have been collapsed.