This Word module should be used for all taxonomic proposals.

Please complete **Part 1** and:

either **Part 3** for proposals to create new taxa or change existing taxa

or **Part 2** for proposals of a general nature.

Submit the completed Word module, together with the accompanying Excel module named in Part 3, to the appropriate ICTV Subcommittee Chair.

The Word module explains and justifies your proposal. The Excel module is a critical document that will be used to implement the proposed taxonomic changes once they are approved and ratified. If proposals presented in the Word module are not presented accurately in the Excel module, the taxonomic changes cannot proceed.

For guidance, see the notes written in blue, below, and the Help Notes in file Taxonomic\_Proposals\_Help\_2019.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Code assigned:** | ***2019.016S*** | |  |
| **Short title:** Create one new species (*Rafivirus C*) in the genus *Rafivirus* | | | |
|  | | | |
| **Author(s) and email address(es):** | | | |
| List authors in a single line *Archives of Virology* citation format (e.g. Smith AB, Huang C-L, Santos, F) | | Provide email address for each author in a single line separated by semi-colons | |
| Zell R, Gorbalenya AE, Hovi T, Knowles NJ, Lindberg M, Oberste S, Palmenberg AC, Reuter G, Simmonds P, Skern T, Tapparel C, Wolthers K, Woo P | | roland.zell@med.uni-jena.de; a.e.gorbalenya@lumc.nl; tapani.hovi@thl.fi; nick.knowles@pirbright.ac.uk; michael.lindberg@lnu.se; soberste@cdc.gov; acpalmen@wisc.edu; reuter.gabor@gmail.com; peter.simmonds@ndm.ox.ac.uk; timothy.skern@meduniwien.ac.at; caroline.tapparel@unige.ch; k.c.wolthers@amc.uva.nl; pcywoo@hkucc.hku.hk | |
| **Author(s) institutional address(es) (optional):**   |  | | --- | | Provide institutional addresses, each on a single line followed by author(s) initials (e.g. University of Woolloomooloo [SAB, HCL]) | | Jena University Hospital [RZ]  Leiden University Medical Center [AEG]  National Institute for Health and Welfare [TH]  The Pirbright Institute [NJK]  Linnaeus University Kalmar [ML]  Centers for Disease Control and Prevention [SO]  University of Wisconsin [ACP]  University of Pécs [GR]  University of Oxford [PS]  Medical University of Vienna [TS]  University of Geneve [CT]  Universiteit van Amsterdam [KW]  University of Hong Kong [PW] | | | | |
| **Corresponding author** | | | |
| **Roland Zell** (roland.zell@med.uni-jena.de) | | | |
| **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) | | ***Picornaviridae* Study Group** | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | |
|  | | | |
|  | | | |
| Date first submitted to ICTV: | | | 21/05/2019 |
| Date of this revision (if different to above): | | |  |

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

**Part 2:** **NON-STANDARD**

Template for any proposal regarding ICTV procedures, rules or policy, not involving the creation of new taxonomy.

| **Text of proposal:** |
| --- |
|  |

**Part 3:** **PROPOSED TAXONOMY**

|  |
| --- |
| **Name of accompanying Excel module:** 2019.016S.A.v1.1newsp\_Rafivirus\_C.xlsx |

The taxonomic changes you are proposing should be presented on an accompanying Excel module, 2019\_TP\_Template\_Excel\_module. Please enter the file name of the completed module in this box.

**Supporting material:**

| additional material in support of this proposal |
| --- |
| 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, please provide a tree where branch length is **proportional to genetic** distance, generated using an appropriate algorithm (Neighbour-Joining, Maximum Likelihood, or Bayesian) and provide evidence of the reliability of the branching (e.g., by bootstrapping).   Please refer to the Help Notes file (Taxonomic\_Proposals\_Help\_2019) for more information. |

**Create 1 new species, *Rafivirus C*, in the existing genus *Rafivirus***

The genus *Rafivirus* presently consists of two species, *Rafivirus A* and *B*. A new picornavirus, named rhimavirus, with similarity to rafiviruses was detected in liver tissue of captured cane toads (*Rhinella marina*) from Australia (Russo et al., 2018). The new virus differs significantly from the known rafiviruses. No virus was isolated yet.

**Relation to rafiviruses and other picornaviruses:**

- Rhimaviruses have a typical picornavirus genome layout but lack a leader protein-encoding sequence:

5'-UTR[1AB-1C-1D/2A-2B-2Chel/3A-3BVPg-3Cpro-3Dpol]3'UTR

(compare Fig. 1 of supporting material)

- Rhimaviruses possess typical hallmarks of picornaviruses:

capsid proteins: 1AB, 1C, 1D have **rhv** domains with drug-binding site,

2Chel: **G**xx**G**x**GKS** motif of helicases,

3BVPg: **Y-3** residue,

3Cpro: **C**x**CG**x14**G**x**H** motif,

3Dpol: **KDE**, **PSG**, **YGDD**, **FLKR** motifs.

- Phylogenetic analyses indicate clustering with rafiviruses of the picornavirus supergroup 2 (*Dicipivirus/Gallivirus/Kobuvirus/Livupivirus/Megrivirus/Oscivirus/Passerivirus/ Poecivirus/Sakobuvirus/Salivirus/Sicinivirus/Rafivirus/Rosavirus*) in the P1 and 3CD trees (compare Figs. 2 & 3 of supporting material).

**Distinguishing features of rhimavirus compared to other rafiviruses:**

- The rhimavirus polyprotein lacks a **leader protein** sequence (compare Fig. 1);

- **1AB protein** of rhimavirus has a C-terminal extension of c. 90 amino acids.

- **2B protein** of rhimavirus has a N-terminal extension of c. 30 amino acids.

- **Divergence** of the rhimavirus polyprotein is greater 40% in comparisons with members of *Rafivirus A* and *B* (compare Table 1). This suggests the existence of a novel rafivirus species.

**Table 1: Divergence of polyprotein sequence**

1 2 3 4

1 Rafivirus A1 [UF4] -

2 Rafivirus A1 [WHWGGF74766] 0.030 -

3 Rafivirus B1 [LPXYC222841] 0.410 0.401 -

4 Rhimavirus [cane toad/AU1/Australia/2017] 0.467 0.469 0.458 -

- **Sequence divergence** (uncorrected p-distances) of orthologous proteins is high in pairwise between-genus comparisons with 31 acknowledged and proposed species of picornavirus supergroup 2. The amino acid divergences range from 64.2 to 83.7% for P1, 65.3-74.3% for 2Chel, 72.6-85.8% for 3Cpro and 48.1-65.2% for 3Dpol (compare Table 2). Divergence to sequences of other picornavirus supergroups is even greater.

**Table 2: Amino acid divergence\***

**rhimavirus vs. member of ... P1 2Chel 3Cpro 3Dpol**

*within-genus* comparisons:

*Rafivirus Rafivirus A* 0.414 0.485 0.597 0.329

*Rafivirus B* 0.403 0.523 0.548 0.327

*between-genus* comparisons:

*Dicipivirus Cadicivirus A* 0.765 0.715 0.726 0.652

*Cadicivirus B* 0.775 0.678 0.763 0.636

*Gallivirus Gallivirus A* 0.836 0.698 0.819 0.497

*Hemipivirus*† *Hemipivirus A*† 0.780 0.713 0.777 0.633

*Kobuvirus Aichivirus A* 0.720 0.677 0.821 0.509

*Aichivirus B* 0.732 0.692 0.801 0.489

*Aichivirus C* 0.718 0.683 0.828 0.493

*Aichivirus D* 0.734 0.743 0.789 0.558

*Aichivirus E* 0.714 0.671 0.773 0.537

*Aichivirus F* 0.728 0.680 0.766 0.508

*Livupivirus Livupivirus A* 0.642 0.653 0.831 0.524

*Ludopivirus*† *Ludopivirus A*† 0.705 0.671 0.823 0.541

*Megrivirus Megrivirus A* 0.811 0.672 0.740 0.602

*Megrivirus B* 0.808 0.666 0.733 0.578

*Megrivirus C* 0.810 0.675 0.803 0.585

*Megrivirus D* 0.806 0.687 0.758 0.599

*Megrivirus E* 0.810 0.669 0.737 0.578

*Myrropivirus*† *Myrropivirus A*† 0.784 0.681 0.759 0.584

*Oscivirus Oscivirus A* 0.749 0.668 0.814 0.499

*Passerivirus Passerivirus A* 0.768 0.719 0.797 0.544

*Passerivirus B* 0.779 0.716 0.808 0.541

*Pemapivirus*† *Pemapivirus A*† 0.779 0.696 0.815 0.539

*Poecivirus Poecivirus A* 0.837 0.717 0.773 0.621

*Rosavirus Rosavirus A* 0.777 0.680 0.758 0.590

*Rosavirus B* 0.777 0.647 0.737 0.601

*Rosavirus C* 0.774 0.654 0.758 0.591

*Sakobuvirus Sakobuvirus A* 0.725 0.712 0.858 0.528

*Salivirus Salivirus A* 0.735 0.667 0.812 0.585

*Sicinivirus Sicinivirus A* 0.805 0.693 0.811 0.481

*Symapivirus*† *Symapivirus A*† 0.805 0.729 0.801 0.590

*Tropivirus*† *Tropivirus A*† 0.788 0.672 0.784 0.634

\* number of amino acid differences per site

† proposed taxa

**Exemplar:**

rafivirus C1 (rhimavirus) strain cane toad/AU1/Australia/2017, GenBank acc. no. MG967619

**Species demarcation criteria:**

Members of a species of the genus *Rafivirus*:

- share a common genome organization,

- share greater than 70% aa identity in the polyprotein,

- share greater than 70% aa identity in the P1,

- share greater than 70% aa identity in the non-structural proteins 2C + 3CD.

| **References:** |
| --- |
| Russo AG, Eden JS, Tuipulotu DE, Shi M, Selechnik D, Shine R, Rollins LA, Holmes EC, White PA. 2018. Viral discovery in the invasive Australian cane toad (*Rhinella marina*) using metatranscriptomic and genomic approaches. J Virol 92:e00768-18. |

**Supporting material:**



**Figure 1:** Schematic depiction of the rafivirus genome organisation. The open reading frames are indicated by boxes. Positions of putative 3Cpro cleavage sites are indicated by ▼. The names and lengths of the deduced proteins are presented. The processing sites at the N- and C-terminus of the 2A protein are unclear.



**Legend to Figure 2:**  Phylogenetic analysis of picornavirus **P1** using Bayesian tree inference (MrBayes 3.2). Eighty picornavirus sequences of the *Dicipivirus/Gallivirus/Kobuvirus/Livupivirus/Megrivirus/Oscivirus/Passerivirus/ Poecivirus/Sakobuvirus/Salivirus/Sicinivirus/Rafivirus/Rosavirus* supergroup were retrieved from GenBank; the entero- and cardiovirus sequences served as outgroup. [Note: the supergroup does not imply a taxonomic entity but reflects phylogenetic clustering of the respective genera observed in different tree inference methods (NJ, ML, Bayesian MCMC).] Presented are GenBank accession numbers, ***genus*** ***names***, *species names*, type and—if available—common names in round brackets. Designations of isolates are given in square brackets. Yet unassigned viruses are printed in blue. The proposed names are printed in red and indicated by a dot (●). Numbers at nodes indicate posterior probabilities obtained after 2,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.



**Legend to Figure 3:**  Phylogenetic analysis of picornavirus **3CD** using Bayesian tree inference (MrBayes 3.2). Seventy-nine picornavirus sequences of the *Dicipivirus/Gallivirus/Kobuvirus/Livupivirus/Megrivirus/Oscivirus/Passerivirus/ Poecivirus/Sakobuvirus/Salivirus/Sicinivirus/Rafivirus/Rosavirus* supergroup were retrieved from GenBank; the entero- and cardiovirus sequences served as outgroup. [Note: the supergroup does not imply a taxonomic entity but reflects phylogenetic clustering of the respective genera observed in different tree inference methods (NJ, ML, Bayesian MCMC).] Presented are GenBank accession numbers, ***genus*** ***names***, *species names*, type and—if available—common names in round brackets. Designations of isolates are given in square brackets. Yet unassigned viruses are printed in blue. The proposed name is printed in red and indicated by a dot (●). Numbers at nodes indicate posterior probabilities obtained after 2,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.