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.

For guidance, see the notes written in blue, below, and the help notes in file Taxonomic\_Proposals\_Help\_2018.

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

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| --- | --- | --- | --- |
| **Code assigned:** | ***2018.010S*** | | (to be completed by ICTV officers) |
| **Short title:** (e.g. “6 new species in the genus *Zetavirus”*)  **1 new picornavirus genus (*Rafivirus*) with 2 species (*Rafivirus A, Rafivirus B*)** | | | |
|  | | | |
| **Author(s):** | | | |
| Roland Zell, Alexander E. Gorbalenya, Tapani Hovi, Andrew M.Q. King, Nick J. Knowles, A. Michael Lindberg, M. Steven Oberste, Ann C. Palmenberg, Gabor Reuter, Peter Simmonds, Tim Skern, Caroline Tapparel, Katja C. Wolthers, Patrick C.Y. Woo | | | |
| **Corresponding author with e-mail address:** | | | |
| Roland Zell ([roland.zell@med.uni-jena.de](mailto: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: | | | 15/06/2018 |
| Date of this revision (if different to above): | | |  |

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| **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:** **2018.010S.N.v1.Rafivirus** |

The taxonomic changes you are proposing should be presented on an accompanying Excel module, 2017\_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, try to provide a tree where branch length is related to genetic distance. |

**Create 2 new species (*Rafivirus A, Rafivirus B*) in a new genus (*Rafivirus*)**

Three novel picornaviruses—named rafiviruses—have been detected in faecal and tissue specimens of Forsten's tortoise (*Indotestudo forstenii*), the Chinese stripe-necked turtle (*Mauremys sinensis*) and a gecko (*Gekko similignum*). No virus was isolated yet. Rafiviruses are diverse picornaviruses of the *Dicipivirus/Gallivirus/Kobuvirus/Megrivirus/Oscivirus/Passerivirus/ Salivirus/Sakobuvirus/Sicinivirus/Rosavirus* supergroup (SG2).

**Relation to other picornaviruses:**

- Rafiviruses have a typical picornavirus genome layout:

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

(compare Fig. 1 of supporting material)

- Rafiviruses 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 a distinct branch that clusters with picornavirus sequences of supergroup 2 (*Aichivirus, Dicipivirus, Gallivirus, Megrivirus, Oscivirus Passerivirus, Rosavirus, Sakobuvirus, Salivirus, Sicinivirus*) in the P1, 2C, 3C, and 3D trees (compare Figs. 2-5 of supporting material).

**Distinguishing features of rafiviruses compared to picornaviruses of SG2:**

1. Rafiviruses A and B have a unique **L protein** each. Only the N-terminal domains (c. 39 aa) of the proposed Rafivirus A and B **L proteins** share similarity.

2. Rafivirus **2A proteins** are not well-defined. They have a length of 43 and 49 aa, respectively, and share little similarity to each other and to other picornavirus proteins.

3. Rafivirus **3A proteins** are rather short (63 aa) but show similarity to the 3A proteins of the remaining SG2 picornaviruses.

4. Rafiviruses have a short 3'-UTR (70 nt and 52 nt, respectively).

4. **Sequence divergence** (uncorrected p-distances) of all relevant genome regions is high in pairwise comparisons with other SG2 picornaviruses (Table 1):

**Table 1: Nucleotide and amino acid divergence**

**P1 2Chel 3Cpro 3Dpol**

**Rafivirus A vs. nt aa nt aa nt aa nt aa**

Rafivirus B 0.369 0.340 0.382 0.411 0.416 0.519 0.312 0.248

Cadicivirus A 0.622 0.784 0.558 0.687 0.596 0.816 0.522 0.656

Gallivirus A 0.659 0.823 0.574 0.686 0.628 0.791 0.475 0.502

Kobuvirus A 0.615 0.710 0.578 0.646 0.690 0.850 0.514 0.507

Megrivirus A 0.629 0.808 0.543 0.656 0.628 0.750 0.496 0.582

Oscivirus A1 0.604 0.759 0.557 0.664 0.607 0.812 0.446 0.497

Passerivirus A 0.652 0.787 0.603 0.723 0.660 0.845 0.505 0.507

Rosavirus A 0.657 0.811 0.572 0.667 0.644 0.801 0.546 0.587

Sakobuvirus A 0.611 0.721 0.587 0.678 0.688 0.867 0.494 0.517

Salivirus A 0.617 0.729 0.588 0.686 0.672 0.853 0.541 0.587

Sicinivirus A 0.648 0.794 0.576 0.682 0.661 0.850 0.494 0.504

**Type species of genus:**

***Rafivirus A***, rafivirus A1 (tortoise rafivirus) [UF4], GenBank acc. no. KJ415177

**Species demarcation criteria:**

Based on available sequence data, preliminary species demarcation criteria were defined.

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.

**Origin of name:**

**rafi** refers to Raph(ael), a fictional tortoise comics character.

| **References:** |
| --- |
| Ng TFF, Wellehan JFX Jr., Goe A, Kondov NO, Reuter G, Wong W, Waltzek TB, Knowles NJ, Delwart E. Chararcterization of a first picornavirus from tortoise. Unpublished.  Shi M, Lin XD, Chen X, tian JH, Chen LJ, Li K, Wang W, Eden JS, Shen JJ, Liu L, Holmes EC, Zhang YZ. 2018. The evolutionary history of vertebrate RNA viruses. Nature 556:197-202. |



**Figure 1:** Schematic depiction of the rafivirus genome organisation (proposed genus: *Rafivirus*). 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). Seventy-five picornavirus sequences of the *Dicipivirus/Gallivirus/Kobuvirus/Megrivirus/Oscivirus/Passerivirus/Salivirus/ Sakobuvirus/Sicinivirus/Rosavirus* supergroup were retrieved from GenBank; the newt ampivirus sequence 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. Proposed names are printed in red and indicated by a dot (●). Numbers at nodes indicate posterior probabilities obtained after 4,000,000 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 **2Chel** using Bayesian tree inference (MrBayes 3.2). Seventy-five picornavirus sequences of the *Dicipivirus/Gallivirus/Kobuvirus/Megrivirus/Oscivirus/Passerivirus/ Salivirus/Sakobuvirus/Sicinivirus/Rosavirus* supergroup were retrieved from GenBank; the newt ampivirus sequence 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. Proposed names are printed in red and indicated by a dot (●). Numbers at nodes indicate posterior probabilities obtained after 4,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.



**Legend to Figure 4:**  Phylogenetic analysis of picornavirus **3Cpro** using Bayesian tree inference (MrBayes 3.2). Seventy-five picornavirus sequences of the *Dicipivirus/Gallivirus/Kobuvirus/Megrivirus/Oscivirus/Passerivirus/ Salivirus/Sakobuvirus/Sicinivirus/Rosavirus* supergroup were retrieved from GenBank; the newt ampivirus sequence 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. Proposed names are printed in red and indicated by a dot (●). Numbers at nodes indicate posterior probabilities obtained after 4,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.



**Legend to Figure 5:**  Phylogenetic analysis of picornavirus **3Dpol** using Bayesian tree inference (MrBayes 3.2). Seventy-five picornavirus sequences of the *Dicipivirus/Gallivirus/Kobuvirus/Megrivirus/Oscivirus/Passerivirus/ Salivirus/Sakobuvirus/Sicinivirus/Rosavirus* supergroup were retrieved from GenBank; the newt ampivirus sequence 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. Proposed names are printed in red and indicated by a dot (●). Numbers at nodes indicate posterior probabilities obtained after 6,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.