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**

|  |  |  |  |
| --- | --- | --- | --- |
| **Code assigned:** | ***2018.005S*** | | (to be completed by ICTV officers) |
| **Short title:** (e.g. “6 new species in the genus *Zetavirus”*)  **1 new picornavirus genus (*Malagasivirus*) with 2 species (*Malagasivirus A* and *Malagasivirus 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): | | |  |

|  |
| --- |
| **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: 2018.005S.N.v1.Malagasivirus** |

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 (*Malagasivirus A* and *Malagasivirus B*) in a new genus (*Malagasivirus*)**

The proposed genus *Malagasivirus* comprises two species each containing one novel picornavirus named lesavirus 1 and 2, respectively. These viruses have been detected in faecal specimens of ring-tailed lemurs (*Lemur catta*) and black-and-white ruffed lemurs (*Varecia variegata*). No virus was isolated yet. Preliminary studies on the prevalence of malagasiviruses in various lemur species revealed an apparent host species-specific distribution:

Lesavirus 1 was only prevalent in faeces of ring-tailed lemurs but not in those of black lemurs, blue-eyed black lemurs, mongoose lemurs, black-and-white ruffed lemurs or Coquerel's sifakas.

Lesavirus 2 was only prevalent in faeces of black-and-white ruffed lemurs but not in those ring-tailed lemurs, black lemurs, blue-eyed black lemurs, mongoose lemurs, or Coquerel's sifakas.

Closest relatives of the malagasiviruses are hunniviruses, teschoviruses and the swine picornavirus Japan Tottori-WOL (compare Figs. 2-5).

**Relation to other picornaviruses:**

- Malagasiviruses have a typical picornavirus genome layout:

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

(compare Fig. 1 of supporting material)

- Malagasiviruses possess typical hallmarks of picornaviruses:

- capsid protein 1B, 1C, 1D: **rhv** domain,

- 2A: **NPGP** sequence motif,

- 2C: **G**xx**G**x**GKS** motif of helicases,

- 3B: **Y** residue in third position,

- 3C: **C**x**CG**x14**G**x**H** motif

- 3D: **KDE**, **PSG**, **YGDD** motifs

- Phylogenetic analyses indicate a distinct branch that clusters either with the unassigned swine picornavirus Tottori-WOL (P1 tree) or the hunniviruses (2Chel, 3Cpro, 3Dpol trees; compare Figs. 2-5 of supporting material).

**Distinguishing features of malagasiviruses compared to hunni- and teschoviruses:**

1. Malagasiviruses have a **unique L protein**.

2. **Sequence divergences** (uncorrected p-distances) of all relevant genome regions is high in pairwise comparisons:

- VP1: nt divergence >0.55, aa divergence >0.65;

- P1: nt divergence >0.47, aa divergence >0.5;

- 2Chel: nt divergence >0.45, aa divergence >0.5;

- 3Cpro: nt divergence >0.5, aa divergence >0.6;

- 3Dpol: nt divergence >0.4, aa divergence >0.42

(compare tables 1-10 of supporting material).

3. The **2B protein** of malagasiviruses shares some similarity to the hunnivirus 2B protein but lacks a C-terminal domain of c. 75-90 aa:

- 157 aa (lesavirus 1) and 158 aa (lesavirus 2) vs. 234-250 aa of hunnivirus 2B;

The malagasivirus **2B protein** shares no similarity to teschovirus 2B.

4. The **3A protein** of malagasiviruses shares little similarity with the respective teschovirus and hunnivirus proteins.

**Distinguishing features of the malagasiviruses A and B:**

1. Malagasiviruses A and B exhibit different host specificities.

2. Sequence divergences (uncorrected p-distances) of all relevant genome regions justify assignment to 2 species:

<52% nt identity in VP1 nt sequence (<50% aai)

<61% nt identity in P1 nt sequence (<61% aai),

<68% nt identity in 2C nt sequence (<76% aai),

<68% nt identity in 3C nt sequence (<73% aai) and

<68% nt identity in 3D nt sequence (<76% aai) (compare tables 1-10 of supporting material).

64.0% nt identity in genome comparison (67.2% aai in the polyprotein).

**Species demarcation criteria:**

Members of a species of genus *Malagasivirus*:

- 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.

**Type species of genus:**

Malagasivirus A, lesavirus 1 [Mis101308/2012], GenBank acc. no. KM396707

**Origin of the names:**

**malagasi** refers to the Madagascan (**Malagas**y) origin of the two lemurs species;

**lesa**virus sigla for **le**mur **s**tool-**a**ssociated virus

| **References:** |
| --- |
| Lim ES, Deem SL, Porton IJ, Cao S, Wang D. 2015. Species-specific transmission of novel picornaviruses in lemurs. J Virol 89(7):4002-4010. |



**Figure 1:** Comparison of the genome organisation of lesavirus, *Hunnivirus A, Teschovirus A* and porcine picornavirus Japan (schematic depiction). The open reading frames are indicated by boxes. Positions of putative 3Cpro cleavage sites are indicated by ▼, the VP0 processing site is indicated by a ¶ and the site of termination/reinitiation of RNA translation at the NPGP sequence motif is indicated by an arrow (↓). The lengths of the deduced proteins are also presented. The 5'-NTRs may be incomplete. Unique genome regions (L, 2B, 3A) are shaded.



**Legend to Figure 2:**  Phylogenetic analyses of picornavirus **P1** using Bayesian tree inference (MrBayes 3.2). Fifty-two picornavirus sequences of the *Aphthovirus/Cardiovirus/Cosavirus/Erbovirus/Hunnivirus/Mischivirus/Mosavirus/ Senecavirus/Teschovirus/Torchivirus* 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*** and—if available—common names and types. 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 analyses of picornavirus **2Chel** using Bayesian tree inference (MrBayes 3.2). Fifty-two picornavirus sequences of the *Aphthovirus/Cardiovirus/Cosavirus/Erbovirus/Hunnivirus/Mischivirus/ Mosavirus/Senecavirus/Teschovirus/Torchivirus* 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*** and—if available—common names and types. 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 2,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.



**Legend to Figure 4:**  Phylogenetic analyses of picornavirus **3Cpro** using Bayesian tree inference (MrBayes 3.2). Fifty-two picornavirus sequences of the *Aphthovirus/Cardiovirus/Cosavirus/Erbovirus/Hunnivirus/Mischivirus/ Mosavirus/Senecavirus/Teschovirus/Torchivirus* 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*** and—if available—common names and types. 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.

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**Legend to Figure 5:**  Phylogenetic analyses of picornavirus **3Dpol** using Bayesian tree inference (MrBayes 3.2). Fifty-two picornavirus sequences of the *Aphthovirus/Cardiovirus/Cosavirus/Erbovirus/Hunnivirus/Mischivirus/ Mosavirus/Senecavirus/Teschovirus/Torchivirus* 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*** and—if available—common names and types. 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 2,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.

**Table 1. Estimates of Evolutionary Divergence between VP1 Nucleotide Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.016 ][0.018 ][0.018 ][0.019 ][0.018 ][0.018 ][0.019 ][0.019 ][0.018 ][0.018 ]

[ 2] 0.482 [0.019 ][0.018 ][0.019 ][0.018 ][0.018 ][0.017 ][0.017 ][0.017 ][0.017 ]

[ 3] 0.586 0.575 [0.018 ][0.018 ][0.018 ][0.018 ][0.018 ][0.018 ][0.019 ][0.018 ]

[ 4] 0.587 0.593 0.352 [0.018 ][0.017 ][0.018 ][0.018 ][0.018 ][0.018 ][0.017 ]

[ 5] 0.580 0.591 0.275 0.343 [0.018 ][0.017 ][0.018 ][0.017 ][0.018 ][0.018 ]

[ 6] 0.608 0.611 0.399 0.409 0.399 [0.019 ][0.019 ][0.019 ][0.016 ][0.017 ]

[ 7] 0.573 0.563 0.471 0.481 0.468 0.455 [0.017 ][0.017 ][0.018 ][0.017 ]

[ 8] 0.576 0.578 0.483 0.478 0.473 0.449 0.287 [0.005 ][0.018 ][0.017 ]

[ 9] 0.572 0.571 0.476 0.480 0.464 0.448 0.287 0.023 [0.017 ][0.017 ]

[10] 0.638 0.649 0.607 0.612 0.600 0.635 0.601 0.610 0.615 [0.017 ]

[11] 0.616 0.607 0.642 0.626 0.639 0.652 0.629 0.628 0.629 0.654

The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 888 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison

**Table 2. Estimates of Evolutionary Divergence between VP1 Amino Acid Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.029 ][0.029 ][0.028 ][0.029 ][0.027 ][0.029 ][0.029 ][0.029 ][0.025 ][0.026 ]

[ 2] 0.520 [0.030 ][0.030 ][0.030 ][0.028 ][0.028 ][0.029 ][0.029 ][0.026 ][0.027 ]

[ 3] 0.697 0.693 [0.028 ][0.025 ][0.031 ][0.032 ][0.032 ][0.032 ][0.028 ][0.028 ]

[ 4] 0.717 0.684 0.321 [0.028 ][0.031 ][0.031 ][0.031 ][0.031 ][0.028 ][0.028 ]

[ 5] 0.706 0.680 0.213 0.274 [0.030 ][0.031 ][0.032 ][0.032 ][0.028 ][0.027 ]

[ 6] 0.740 0.707 0.385 0.412 0.406 [0.032 ][0.032 ][0.032 ][0.026 ][0.027 ]

[ 7] 0.715 0.719 0.526 0.561 0.531 0.487 [0.026 ][0.026 ][0.028 ][0.027 ]

[ 8] 0.706 0.698 0.507 0.548 0.511 0.450 0.203 [0.006 ][0.028 ][0.027 ]

[ 9] 0.699 0.696 0.502 0.544 0.507 0.454 0.207 0.009 [0.027 ][0.028 ]

[10] 0.790 0.776 0.747 0.754 0.752 0.771 0.760 0.763 0.770 [0.024 ]

[11] 0.784 0.764 0.790 0.779 0.795 0.801 0.798 0.796 0.794 0.824

The number of amino acid differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 amino acid sequences. The coding data was translated assuming a Standard genetic code table. All ambiguous positions were removed for each sequence pair. There were a total of 296 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison

**Table 3. Estimates of Evolutionary Divergence between P1 Nucleotide Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.009 ][0.009 ][0.009 ][0.009 ][0.010 ][0.009 ][0.010 ][0.010 ][0.009 ][0.009 ]

[ 2] 0.398 [0.009 ][0.010 ][0.010 ][0.010 ][0.009 ][0.010 ][0.010 ][0.011 ][0.009 ]

[ 3] 0.492 0.486 [0.008 ][0.008 ][0.009 ][0.009 ][0.009 ][0.009 ][0.011 ][0.010 ]

[ 4] 0.499 0.502 0.282 [0.008 ][0.010 ][0.010 ][0.010 ][0.009 ][0.010 ][0.010 ]

[ 5] 0.496 0.486 0.253 0.280 [0.009 ][0.009 ][0.010 ][0.010 ][0.011 ][0.010 ]

[ 6] 0.503 0.499 0.355 0.356 0.347 [0.009 ][0.009 ][0.009 ][0.010 ][0.009 ]

[ 7] 0.499 0.500 0.394 0.400 0.377 0.379 [0.008 ][0.008 ][0.010 ][0.009 ]

[ 8] 0.482 0.502 0.392 0.399 0.376 0.380 0.216 [0.004 ][0.010 ][0.009 ]

[ 9] 0.481 0.499 0.387 0.397 0.374 0.378 0.221 0.039 [0.010 ][0.009 ]

[10] 0.558 0.556 0.542 0.556 0.533 0.554 0.538 0.546 0.548 [0.010 ]

[11] 0.520 0.528 0.546 0.539 0.547 0.545 0.537 0.535 0.539 0.573

The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 2745 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison

**Table 4. Estimates of Evolutionary Divergence between P1 Amino Acid Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.017 ][0.016 ][0.016 ][0.016 ][0.016 ][0.016 ][0.017 ][0.017 ][0.016 ][0.016 ]

[ 2] 0.397 [0.017 ][0.017 ][0.017 ][0.017 ][0.016 ][0.016 ][0.016 ][0.017 ][0.015 ]

[ 3] 0.560 0.538 [0.013 ][0.011 ][0.016 ][0.016 ][0.016 ][0.016 ][0.016 ][0.017 ]

[ 4] 0.565 0.537 0.187 [0.013 ][0.016 ][0.016 ][0.016 ][0.016 ][0.017 ][0.016 ]

[ 5] 0.556 0.537 0.149 0.160 [0.016 ][0.016 ][0.017 ][0.017 ][0.017 ][0.016 ]

[ 6] 0.576 0.550 0.302 0.313 0.307 [0.016 ][0.016 ][0.016 ][0.016 ][0.016 ]

[ 7] 0.553 0.538 0.368 0.378 0.368 0.353 [0.011 ][0.011 ][0.016 ][0.017 ]

[ 8] 0.543 0.528 0.361 0.381 0.361 0.346 0.126 [0.003 ][0.016 ][0.017 ]

[ 9] 0.536 0.525 0.357 0.377 0.355 0.346 0.125 0.008 [0.016 ][0.017 ]

[10] 0.667 0.646 0.639 0.648 0.639 0.642 0.642 0.648 0.649 [0.015 ]

[11] 0.591 0.595 0.633 0.632 0.637 0.630 0.636 0.625 0.624 0.694

Table. Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 amino acid sequences. The coding data was translated assuming a Standard genetic code table. All ambiguous positions were removed for each sequence pair. There were a total of 915 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison

**Table 5. Estimates of Evolutionary Divergence between 2Chel Nucleotide Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.014 ][0.016 ][0.016 ][0.015 ][0.015 ][0.016 ][0.015 ][0.015 ][0.015 ][0.016 ]

[ 2] 0.324 [0.014 ][0.015 ][0.015 ][0.015 ][0.015 ][0.015 ][0.015 ][0.016 ][0.016 ]

[ 3] 0.460 0.461 [0.013 ][0.014 ][0.014 ][0.014 ][0.014 ][0.014 ][0.015 ][0.016 ]

[ 4] 0.476 0.458 0.226 [0.014 ][0.014 ][0.014 ][0.014 ][0.014 ][0.015 ][0.016 ]

[ 5] 0.470 0.475 0.278 0.286 [0.013 ][0.013 ][0.013 ][0.013 ][0.015 ][0.016 ]

[ 6] 0.481 0.472 0.296 0.281 0.226 [0.009 ][0.010 ][0.010 ][0.015 ][0.016 ]

[ 7] 0.471 0.464 0.288 0.271 0.239 0.124 [0.010 ][0.009 ][0.015 ][0.016 ]

[ 8] 0.470 0.468 0.283 0.279 0.224 0.126 0.117 [0.005 ][0.015 ][0.015 ]

[ 9] 0.460 0.471 0.287 0.276 0.221 0.125 0.107 0.028 [0.015 ][0.015 ]

[10] 0.465 0.498 0.483 0.470 0.452 0.479 0.482 0.483 0.482 [0.015 ]

[11] 0.538 0.536 0.537 0.523 0.542 0.537 0.535 0.545 0.540 0.565

The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 1011 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison

**Table 6. Estimates of Evolutionary Divergence between 2Chel Amino Acid Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.023 ][0.027 ][0.027 ][0.027 ][0.027 ][0.027 ][0.027 ][0.027 ][0.027 ][0.026 ]

[ 2] 0.245 [0.026 ][0.027 ][0.027 ][0.027 ][0.027 ][0.027 ][0.027 ][0.027 ][0.026 ]

[ 3] 0.542 0.533 [0.016 ][0.022 ][0.023 ][0.023 ][0.022 ][0.022 ][0.027 ][0.026 ]

[ 4] 0.536 0.536 0.090 [0.022 ][0.023 ][0.022 ][0.023 ][0.022 ][0.027 ][0.026 ]

[ 5] 0.537 0.547 0.217 0.207 [0.021 ][0.021 ][0.021 ][0.021 ][0.027 ][0.025 ]

[ 6] 0.547 0.566 0.232 0.232 0.180 [0.010 ][0.012 ][0.011 ][0.027 ][0.025 ]

[ 7] 0.537 0.559 0.214 0.211 0.173 0.037 [0.009 ][0.009 ][0.027 ][0.025 ]

[ 8] 0.534 0.553 0.207 0.211 0.183 0.053 0.028 [0.004 ][0.027 ][0.026 ]

[ 9] 0.531 0.550 0.207 0.211 0.176 0.046 0.028 0.006 [0.027 ][0.026 ]

[10] 0.585 0.573 0.586 0.567 0.561 0.580 0.574 0.574 0.571 [0.026 ]

[11] 0.669 0.667 0.633 0.633 0.630 0.655 0.646 0.642 0.639 0.650

Table. Estimates of Evolutionary Divergence between Sequences

The number of amino acid differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 amino acid sequences. The coding data was translated assuming a Standard genetic code table. All ambiguous positions were removed for each sequence pair. There were a total of 337 positions in **the final datase**t. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison

**Table 7. Estimates of Evolutionary Divergence between 3Cpro Nucleotide Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.017 ][0.019 ][0.019 ][0.019 ][0.019 ][0.019 ][0.018 ][0.019 ][0.019 ][0.020 ]

[ 2] 0.321 [0.020 ][0.020 ][0.020 ][0.019 ][0.019 ][0.019 ][0.019 ][0.019 ][0.020 ]

[ 3] 0.545 0.519 [0.015 ][0.014 ][0.016 ][0.016 ][0.016 ][0.016 ][0.019 ][0.020 ]

[ 4] 0.553 0.496 0.207 [0.015 ][0.017 ][0.016 ][0.016 ][0.016 ][0.019 ][0.021 ]

[ 5] 0.556 0.529 0.160 0.229 [0.015 ][0.015 ][0.015 ][0.015 ][0.019 ][0.020 ]

[ 6] 0.564 0.548 0.313 0.316 0.281 [0.011 ][0.011 ][0.011 ][0.020 ][0.020 ]

[ 7] 0.558 0.529 0.311 0.293 0.272 0.096 [0.009 ][0.009 ][0.020 ][0.020 ]

[ 8] 0.554 0.537 0.293 0.298 0.258 0.100 0.067 [0.007 ][0.020 ][0.019 ]

[ 9] 0.563 0.534 0.295 0.300 0.258 0.103 0.071 0.037 [0.020 ][0.019 ]

[10] 0.543 0.554 0.577 0.590 0.567 0.573 0.574 0.569 0.569 [0.019 ]

[11] 0.593 0.603 0.593 0.557 0.595 0.586 0.583 0.602 0.592 0.599

The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 648 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison

**Table 8. Estimates of Evolutionary Divergence between 3Cpro Amino Acid Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.030 ][0.032 ][0.033 ][0.032 ][0.032 ][0.033 ][0.033 ][0.033 ][0.032 ][0.030 ]

[ 2] 0.271 [0.032 ][0.033 ][0.032 ][0.033 ][0.033 ][0.033 ][0.033 ][0.032 ][0.030 ]

[ 3] 0.644 0.642 [0.023 ][0.022 ][0.030 ][0.029 ][0.029 ][0.029 ][0.032 ][0.032 ]

[ 4] 0.624 0.637 0.149 [0.025 ][0.031 ][0.030 ][0.030 ][0.030 ][0.032 ][0.032 ]

[ 5] 0.653 0.657 0.125 0.173 [0.028 ][0.027 ][0.028 ][0.027 ][0.032 ][0.032 ]

[ 6] 0.657 0.655 0.266 0.285 0.237 [0.014 ][0.016 ][0.016 ][0.031 ][0.031 ]

[ 7] 0.649 0.647 0.260 0.264 0.221 0.048 [0.010 ][0.009 ][0.031 ][0.031 ]

[ 8] 0.649 0.647 0.255 0.255 0.226 0.063 0.024 [0.005 ][0.031 ][0.031 ]

[ 9] 0.653 0.652 0.260 0.260 0.221 0.058 0.019 0.005 [0.031 ][0.031 ]

[10] 0.640 0.663 0.682 0.697 0.692 0.700 0.701 0.711 0.706 [0.029 ]

[11] 0.745 0.754 0.695 0.680 0.695 0.709 0.705 0.710 0.705 0.773

The number of amino acid differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 amino acid sequences. The coding data was translated assuming a Standard genetic code table. All ambiguous positions were removed for each sequence pair. There were a total of 216 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison

**Table 9. Estimates of Evolutionary Divergence between 3Dpol Nucleotide Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.012 ][0.014 ][0.013 ][0.013 ][0.013 ][0.013 ][0.013 ][0.013 ][0.013 ][0.014 ]

[ 2] 0.310 [0.013 ][0.012 ][0.012 ][0.013 ][0.013 ][0.013 ][0.013 ][0.015 ][0.014 ]

[ 3] 0.425 0.414 [0.010 ][0.010 ][0.011 ][0.011 ][0.011 ][0.011 ][0.013 ][0.013 ]

[ 4] 0.410 0.419 0.156 [0.010 ][0.010 ][0.010 ][0.009 ][0.009 ][0.014 ][0.014 ]

[ 5] 0.420 0.415 0.189 0.173 [0.010 ][0.011 ][0.011 ][0.010 ][0.013 ][0.014 ]

[ 6] 0.415 0.422 0.200 0.160 0.212 [0.006 ][0.006 ][0.006 ][0.014 ][0.014 ]

[ 7] 0.422 0.419 0.216 0.166 0.222 0.056 [0.006 ][0.006 ][0.014 ][0.014 ]

[ 8] 0.420 0.423 0.207 0.162 0.220 0.057 0.060 [0.003 ][0.014 ][0.013 ]

[ 9] 0.423 0.427 0.211 0.159 0.215 0.056 0.060 0.018 [0.014 ][0.014 ]

[10] 0.483 0.478 0.481 0.480 0.486 0.477 0.477 0.470 0.474 [0.013 ]

[11] 0.467 0.452 0.473 0.470 0.459 0.465 0.467 0.466 0.464 0.527

The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 1392 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison

**Table 10. Estimates of Evolutionary Divergence between 3Dpol Amino Acid Sequences**

[ 1] #KM396707 Lesavirus 1 [Mis101308/2012]

[ 2] #KM396708 Lesavirus 2 [Nai108015/2012]

[ 3] #JQ941880 Hunnivirus A1 (bovine hungarovirus) [BHUV1/2008/HUN]

[ 4] #HM153767 Hunnivirus A2 (ovine\_hungarovirus) [OHUV1/2009/HUN]

[ 5] #KX156157 Hunnivirus A6 [rodent/Rn/PicoV/SX2015\_2]

[ 6] #KJ950971 Hunnivirus A4 (Norway rat hunnivirus) [NrHuV/NYC-E21]

[ 7] #KT944214 Hunnivirus A7 [05VZ-75-RAT099]

[ 8] #KT944212 Hunnivirus A8 [83GR-70-RAT106]

[ 9] #KT944213 Hunnivirus A8 [83GR-70-RAT130]

[10] #AF231769 Teschovirus A1 PTV1 [Talfan]

[11] #LC113907 Swine\_picornavirus Japan [Tottori-WOL]

[ 1 2 3 4 5 6 7 8 9 10 11 ]

[ 1] [0.019 ][0.022 ][0.022 ][0.022 ][0.022 ][0.022 ][0.021 ][0.022 ][0.023 ][0.022 ]

[ 2] 0.243 [0.022 ][0.022 ][0.022 ][0.022 ][0.022 ][0.022 ][0.022 ][0.023 ][0.022 ]

[ 3] 0.432 0.434 [0.012 ][0.014 ][0.014 ][0.015 ][0.014 ][0.014 ][0.023 ][0.022 ]

[ 4] 0.425 0.432 0.081 [0.013 ][0.013 ][0.013 ][0.013 ][0.013 ][0.023 ][0.022 ]

[ 5] 0.439 0.436 0.110 0.094 [0.015 ][0.015 ][0.015 ][0.015 ][0.023 ][0.022 ]

[ 6] 0.431 0.433 0.116 0.088 0.121 [0.007 ][0.007 ][0.007 ][0.023 ][0.022 ]

[ 7] 0.432 0.428 0.125 0.094 0.132 0.024 [0.009 ][0.008 ][0.023 ][0.022 ]

[ 8] 0.430 0.434 0.121 0.092 0.125 0.029 0.039 [0.004 ][0.023 ][0.022 ]

[ 9] 0.430 0.430 0.123 0.090 0.123 0.026 0.037 0.007 [0.023 ][0.022 ]

[10] 0.524 0.531 0.517 0.512 0.530 0.511 0.510 0.514 0.514 [0.023 ]

[11] 0.491 0.478 0.492 0.484 0.503 0.498 0.503 0.501 0.503 0.579

The number of amino acid differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 11 amino acid sequences. The coding data was translated assuming a Standard genetic code table. All ambiguous positions were removed for each sequence pair. There were a total of 464 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

\_\_\_ within type comparison, \_\_\_ between types/within species comparison,

\_\_\_ between species/within genus comparison, \_\_\_ between genera comparison