



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:	2016.001aS	(to be completed by ICTV officers)
Short title: Create 3 new species (<i>Aichivirus D, E, F</i>) in the genus <i>Kobuvirus</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)		
Modules attached (modules 1 and 11 are required)	6 <input type="checkbox"/> 7 <input type="checkbox"/> 8 <input type="checkbox"/> 9 <input type="checkbox"/> 10 <input type="checkbox"/> 11 <input checked="" type="checkbox"/> 2 <input checked="" type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5 <input type="checkbox"/>	

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)

Picornaviridae Study Group

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

Date first submitted to ICTV:

15/06/2016

Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

MODULE 2: NEW SPECIES

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be “unassigned” within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	2016.001aS	(assigned by ICTV officers)
To create 3 new species within:		
Genus:	<i>Kobuvirus</i>	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.
Subfamily:		
Family:	<i>Picornaviridae</i>	
Order:	<i>Picornavirales</i>	
Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
<i>Aichivirus D</i>	kagovirus 1 [Kagoshima-1-22-KoV/2014/Jpn]	LC055961
<i>Aichivirus E</i>	rabbit picornavirus [Rabbit01/2013/HUN]	KT325853
<i>Aichivirus F</i>	bat kobuvirus [BtMr-PicoV/JX2010]	KJ641686

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 11

Novel picornaviruses have been detected in faecal samples of diarrheic bovine calves (Japanese black calves) in Japan, clinically healthy rabbits in Hungary and in bats (*Myotis ricketti*) in China. The proposed kobuvirus species share significant similarities to *Kobuvirus*, i.e.

(i) identical genome layout:

VPg+5'UTR^{IR_{ES}-V}[L-1AB-1C-1D/2A^{H-Box/NC}-2B-2C^{Hel}/3A-3B^{VPg}-3C^{Pro}-3D^{Pol}]3'UTR-poly(A);

(ii) significant amino acid identities of capsid proteins (50-64%, compare Appendix Table 1) and 3CD (>52-69%, compare Appendix Table 2); amino acid identities of whole genomes: ≥50%

(iii) cluster with kobuviruses in phylogenetic trees (compare Appendix Figures 1-4).

MODULE 11: **APPENDIX**: supporting material

additional material in support of this proposal

References:

- Pankovics P, Boros A, Biro H, Horvath KB, Phan TG, Delwart E, Reuter G. 2015. Novel picornavirus in domestic rabbits (*Oryctolagus cuniculus var. domestica*). Infect. Genet. Evol. 37:117-122.
- Otomaru K, Naoi Y, Haga K, Omatsu T, Uto T, Koizumi M, Masuda T, Yamasato H, Takai H, Aoki H, Tsuchiaka S, Sano K, Okazaki S, Katayama Y, Oba M, Furuya T, Shirai J, Katayama K, Mizutani T, Nagai M. 2016. Detection of novel kobu-like viruses in Japanese black cattle in Japan. J. Vet. Med. Sci. 78(2):321-324.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

Fig. 1
P1

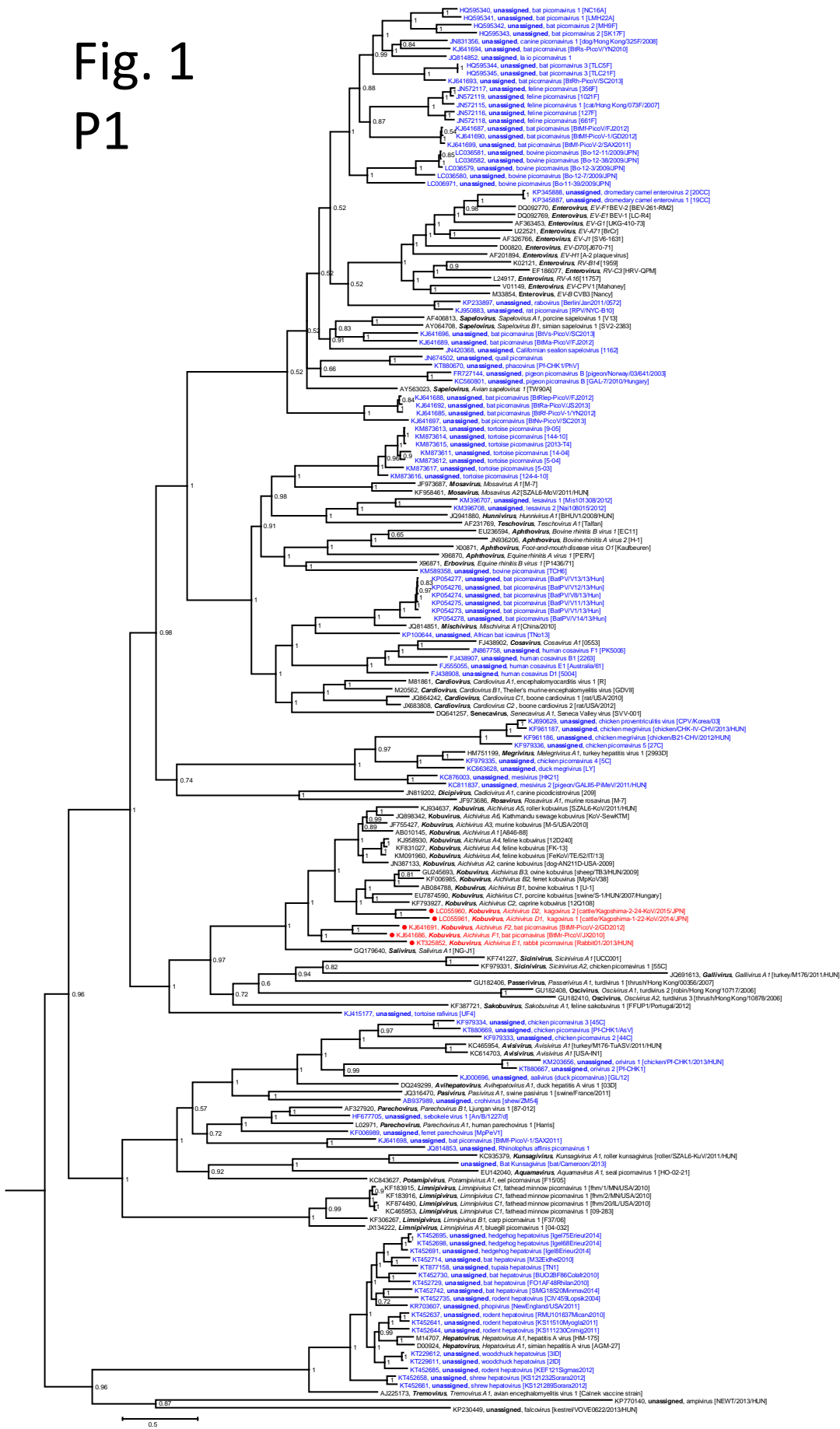


Figure 1: Phylogenetic analyses of picornavirus P1 gene regions using Bayesian tree inference (MrBayes 3.2). 178 picornavirus sequences were retrieved from GenBank. Presented are GenBank accession numbers, ***genus names***, *species names* and *types*. If available, designations of isolates are given 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.

3CD



Figure 2: Phylogenetic analyses of picornavirus 3CD gene regions of picornaviruses using Bayesian tree inference (MrBayes 3.2). 178 sequences were retrieved from GenBank. Presented are GenBank accession numbers, *genus*, *species names* and *types*. If available, designations of isolates and sequenced specimens, respectively, 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 4,750,000 generations (**B**). The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.

Fig. 3
P1

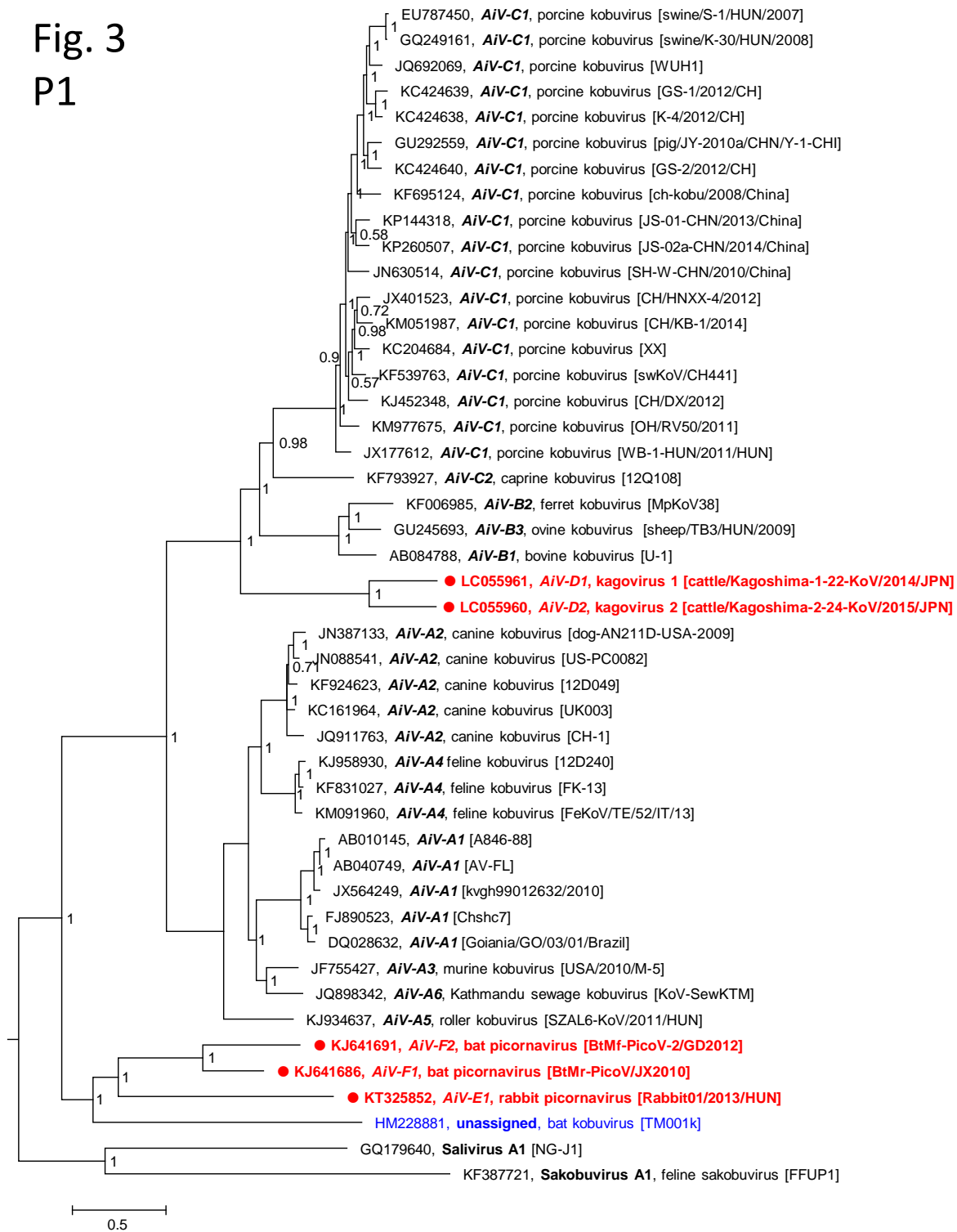


Figure 3: Phylogenetic analyses of *Kobuvirus* P1 using Bayesian tree inference (MrBayes 3.2). 44 kobuvirus sequences, 1 *Salivirus* and 1 *Sakobuvirus* sequence (outgroup) were retrieved from GenBank. Proposed names are printed in red and indicated by a dot (●). The unassigned virus is printed in blue. Presented are Gnk accession numbers, *genus name*, *species names* and *types*. If available, designations of isolates are given in square brackets. Numbers at nodes indicate posterior probabilities obtained after 1,000,000 generations. The optimal substitution model (GTR+G) was determined with MEGA 5. The scale indicates substitutions/site. Abbreviation: AiV, *Aichivirus*.

Fig. 4
3CD

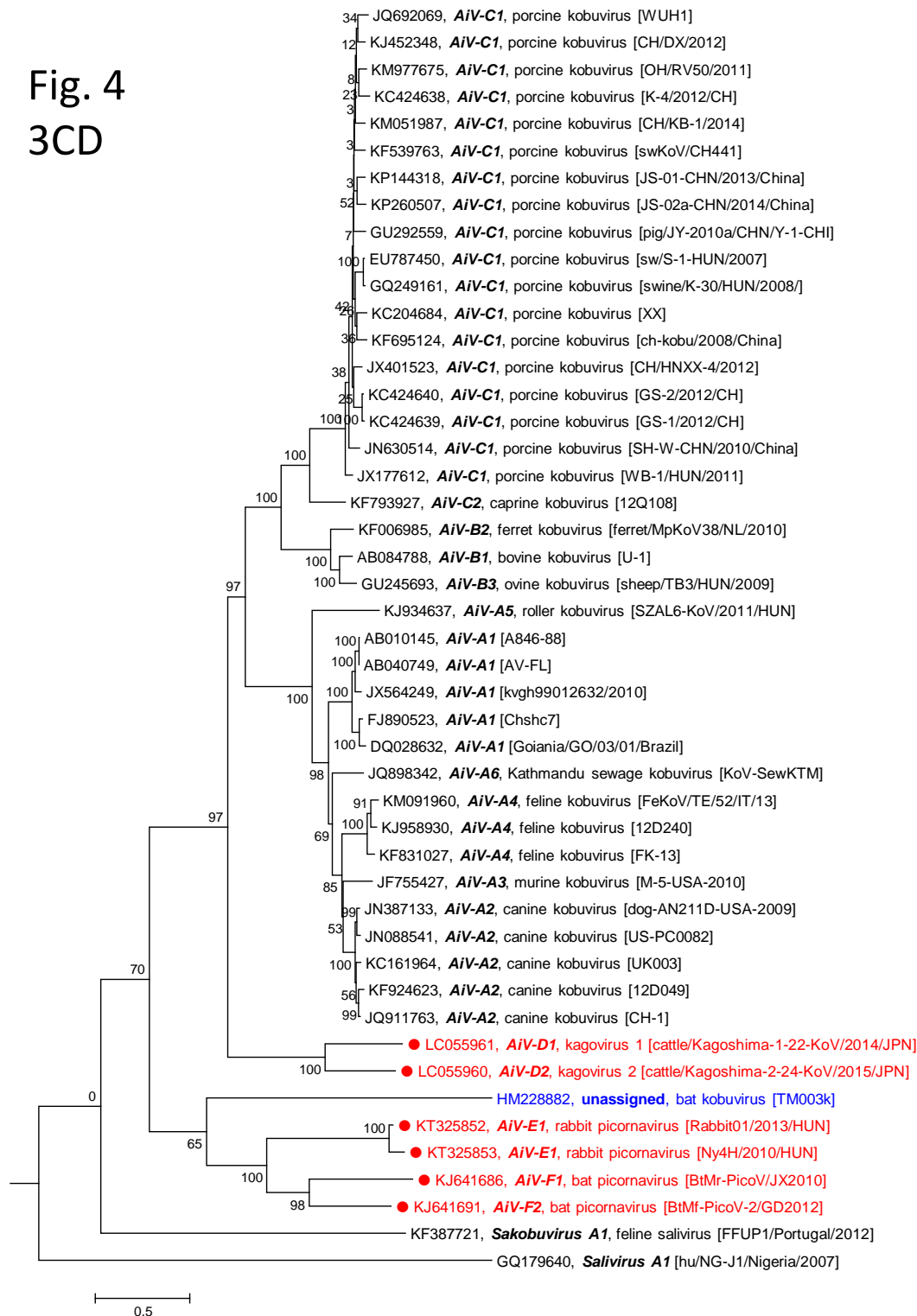


Figure 4: Phylogenetic analyses of *Kobuvirus* 3CD using Maximum likelihood tree inference (MEGA5.2). 45 kobuvirus sequences, 1 *Salivirus* and 1 *Sakobuvirus* sequence (outgroup) were retrieved from GenBank. Proposed names are printed in red and indicated by a dot (●). The unassigned virus is printed in blue. Presented are GenBank accession numbers, **genus name**, **species names** (*Aichivirus* A-F, *AiV*-A to -F) and type designations. If available, designations of isolates are given in square brackets. Numbers at nodes indicate posterior probabilities obtained after 1,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site. Abbreviation: *AiV*, *Aichivirus*.

Table 1: Estimates of evolutionary divergence between P1 aa sequences:

Title:	
Description	
Analysis	
Analysis -----	Distance Estimation
Scope -----	Pairs of taxa
Estimate Variance	
Variance Estimation Method -----	None
Substitution Model	
Substitutions Type -----	Amino acid
Genetic Code Table -----	Standard
Model/Method -----	p-distance
Substitutions to Include -----	All
Rates and Patterns	
Rates among Sites -----	Uniform rates
Pattern among Lineages -----	Same (Homogeneous)
Data Subset to Use	
Gaps/Missing Data Treatment -----	Pairwise deletion
Codons Included -----	1st+2nd+3rd+Non-Coding
No. of Sites : 955	
d : Estimate	
[1] Aichivirus_A1_AB010145_A846-88	
[2] Aichivirus_A1_AB040749_AV-FL	
[3] Aichivirus_A1_FJ890523_isolate_Chshc7	
[4] Aichivirus_A1_DQ028632_isolate_Goiania/GO/03/01/Brazil	
[5] Aichivirus_A1_JX564249_strain_kvgh99012632/2010	
[6] Aichivirus_A2_JN387133_CKV_dog-AN211D-USA-2009	
[7] Aichivirus_A2_KF924623_CKV_isolate_12D049	
[8] Aichivirus_A2_KC161964_CKV_strain_UK003	
[9] Aichivirus_A2_JQ911763_CKV_CH-1	
[10] Aichivirus_A2_JN088541_CKV_US-PC0082	
[11] Aichivirus_A3_JF755427_MKV_M-5/USA/2010_M-5	
[12] Aichivirus_A4_KJ958930_FKV_isolate_12D240	
[13] Aichivirus_A4_KM091960_FKV_strain_FeKoV/TE/52/IT/13	
[14] Aichivirus_A4_KF831027_FKV_strain_FK-13	
[15] Aichivirus_A5_KJ934637_roller_kobuvirus_SZAL6-KoV/2011/HUN	
[16] Aichivirus_A6_JQ898342_sewage_Kathmandu_isolate_KoV-SewKTM	
[17] Aichivirus_B1_AB084788_BKV-1_U-1	
[18] Aichivirus_B2_KF006985_Ferret_kobuvirus_isolate_MpKoV38	
[19] Aichivirus_B3_GU245693_Kobuvirus_sheep/TB3/HUN/2009	
[20] Aichivirus_C1_EU787450_PKV_swine-S-1-HUN-2007-Hungary	
[21] Aichivirus_C1_KC204684_Porcine_kobuvirus_isolate_XX	
[22] Aichivirus_C1_JX401523_Porcine_kobuvirus_isolate_CH/HNXX-4/2012	
[23] Aichivirus_C1_GU292559_Kobuvirus_pig/JY-2010a/CHN_isolate_Y-1-CHI	
[24] Aichivirus_C1_KM051987_Porcine_kobuvirus_strain_CH/KB-1/2014	
[25] Aichivirus_C1_KP144318_Porcine_kobuvirus_JS-01-CHN/2013/China	
[26] Aichivirus_C1_KF539763_Porcine_kobuvirus_isolate_swKoV_CH441	
[27] Aichivirus_C1_JQ692069_Porcine_kobuvirus_strain_WUH1	
[28] Aichivirus_C1_KM977675_Porcine_kobuvirus_isolate_OH/RV50/2011	
[29] Aichivirus_C1_KJ452348_Porcine_kobuvirus_isolate_CH/DX/2012	
[30] Aichivirus_C1_GQ249161_Porcine_kobuvirus_swine/K-30-HUN/2008/HUN	
[31] Aichivirus_C1_KC424640_Porcine_kobuvirus_isolate_GS-2/2012/CH	
[32] Aichivirus_C1_KC424639_Porcine_kobuvirus_isolate_GS-1/2012/CH	
[33] Aichivirus_C1_KC424638_Porcine_kobuvirus_isolate_K-4/2012/CH	
[34] Aichivirus_C1_JX177612_Porcine_kobuvirus_strain_WB-1-HUN/2011/HUN	
[35] Aichivirus_C1_JN630514_Porcine_kobuvirus_SH-W-CHN/2010/China	

[36] Aichivirus_C1_KP260507_Porcine_kobuvirus_isolate_JS-02a-CHN/2014/China
[37] Aichivirus_C1_KF695124_Porcine_kobuvirus_isolate_ch-kobu/2008/China
[38] Aichivirus_C2_KF793927_Caprine_kobuvirus_isolate_12Q108
[39] Aichivirus_D1_LC055961_Kagovirus_cattle/Kagoshima-1-22-KoV/2014/JPN
[40] Aichivirus_D2_LC055960_Kagovirus_cattle/Kagoshima-2-24-KoV/2015/JPN
[41] AiV-E1_KT325852_Rabbit_picornavirus_strain_Rabbit01/2013/HUN
[42] AiV-F2_KJ641691_Bat_picornavirus_isolate_BtMf-PicoV-2/GD2012
[43] AiV-F1_KJ641686_Bat_picornavirus_isolate_BtMr-PicoV/JX2010
[44] Salivirus_A1_GQ179640_NG-J1
[45] SakV-A1_KF387721_Feline_sakobuvirus_A_isolate_FFUP1

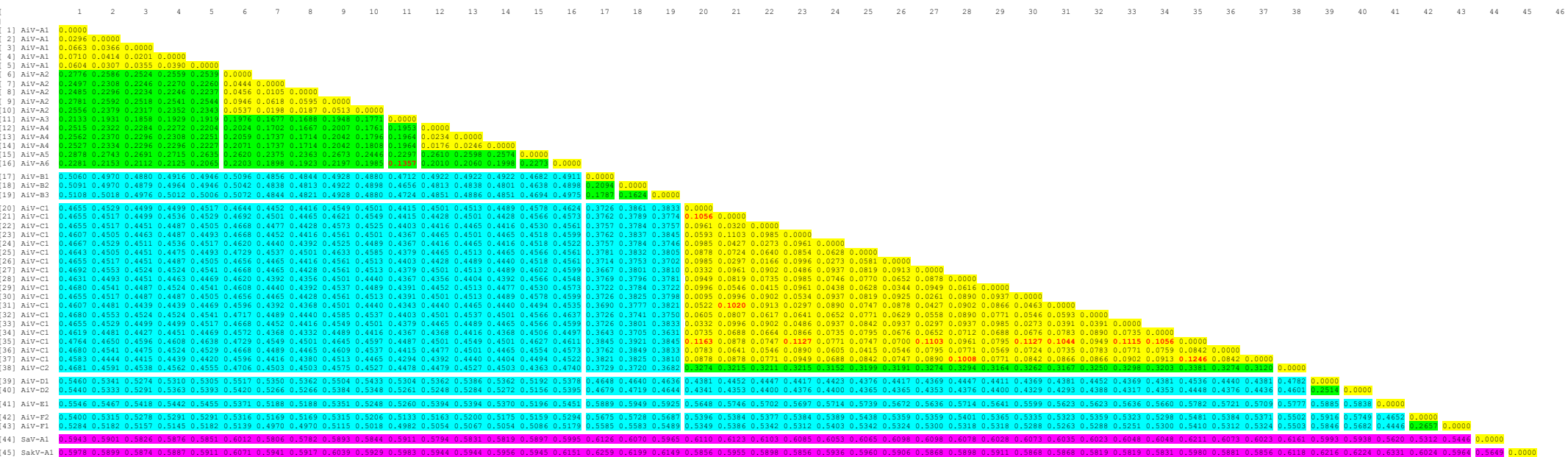


Table. Estimates of Evolutionary Divergence between Sequences
The number of amino acid differences per site from between sequences are shown. The analysis involved 46 amino acid sequences. The coding data was translated assuming a Standard genetic code table. All ambiguous positions were removed for each sequence pair. There were a total of 955 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

I. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution 28: 2731-2739.

P1:	intra-typic	observed aa divergence: <13%	⇒	aa identity: >85%
	inter-typic (within species)	observed aa divergence: 14-34%	⇒	aa identity: 66-84%
	between species	observed aa divergence: 36-50%	⇒	aa identity: 50-64%
	between genera	observed aa divergence: 53-63%	⇒	aa identity: 37-47%

Table 2: Estimates of evolutionary divergence between 3CD aa sequences:

Title:	
Description	
Analysis	
Analysis -----	Distance Estimation
Scope -----	Pairs of taxa
Estimate Variance	
Variance Estimation Method -----	None
Substitution Model	
Substitutions Type -----	Amino acid
Genetic Code Table -----	Standard
Model/Method -----	p-distance
Substitutions to Include -----	All
Rates and Patterns	
Rates among Sites -----	Uniform rates
Pattern among Lineages -----	Same (Homogeneous)
Data Subset to Use	
Gaps/Missing Data Treatment -----	Pairwise deletion
Codons Included -----	1st+2nd+3rd+Non-Coding
No. of Sites : 675	
d : Estimate	
[1] AiV-A1_AB010145_A846-88	
[2] AiV-A1_FJ890523_isolate_Chshc7	
[3] AiV-A1_DQ028632_isolate_Goiania/GO/03/01/Brazil	
[4] AiV-A1_AB040749_AV-FL	
[5] AiV-A1_JX564249_strain_kvgh99012632/2010	
[6] AiV-A2_JN387133_CKV-1_dog-AN211D-USA-2009	
[7] AiV-A2_KF924623_Canine_kobuvirus_1_isolate_12D049	
[8] AiV-A2_KC161964_Canine_kobuvirus_strain_UK003	
[9] AiV-A2_JQ911763_Canine_kobuvirus_CH-1	
[10] AiV-A2_JN088541_Canine_kobuvirus_US-PC0082	
[11] AiV-A3_JF755427_MKV-1_M-5-USA-2010	
[12] AiV-A4_KM091960_Feline_kobuvirus_strain_FeKoV/TE/52/IT/13	
[13] AiV-A4_KF831027_Feline_kobuvirus_strain_FK-13	
[14] AiV-A4_KJ958930_Feline_kobuvirus_isolate_12D240	
[15] AiV-A5_KJ934637_roller_kobuvirus_SZAL6-KoV/2011/HUN	
[16] AiV-A6_JQ898342_Kobuvirus_sewage_Kathmandu_isolate_KoV-SewKTM_cg	
[17] AiV-D1_LC055961_Kagovirus_cattle/Kagoshima-1-22-KoV/2014/JPN	
[18] AiV-D2_LC055960_Kagovirus_cattle/Kagoshima-2-24-KoV/2015/JPN	
[19] AiV-B1_AB084788_BKV-1_U-1	
[20] AiV-B2_KF006985_Ferret_kobuvirus_isolate_ferret/MpKoV38/NL/2010	
[21] AiV-B3_GU245693_sheep/TB3/HUN/2009	
[22] AiV-C1_EU787450_PKV-1_sw/S-1-HUN/2007	
[23] AiV-C1_KC204684_PKV-1_isolate_XX	
[24] AiV-C1_JX401523_PKV-1_isolate_CH/HNXX-4/2012	
[25] AiV-C1_GU292559_PKV-1_pig/JY-2010a/CHN_isolate_Y-1-CHI	
[26] AiV-C1_KM051987_PKV-1_strain_CH/KB-1/2014	
[27] AiV-C1_KP144318_PKV-1_JS-01-CHN/2013/China	
[28] AiV-C1_KF539763_PKV-1_isolate_swKoV_CH441	
[29] AiV-C1_JQ692069_PKV-1_strain_WUH1	
[30] AiV-C1_KM977675_PKV-1_isolate_OH/RV50/2011	
[31] AiV-C1_KJ452348_PKV-1_isolate_CH/DX/2012	
[32] AiV-C1_GQ249161_PKV-1_swine/K-30-HUN/2008/HUN	
[33] AiV-C1_KC424640_PKV-1_isolate_GS-2/2012/CH	
[34] AiV-C1_KC424639_PKV-1_isolate_GS-1/2012/CH	
[35] AiV-C1_KC424638_PKV-1_isolate_K-4/2012/CH	
[36] AiV-C1_JX177612_PKV-1_strain_WB-1-HUN/2011/HUN	

[illegible]

The number of amino acid differences per site from between sequences are shown. The analysis involved 46 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 675 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [1].

3CD:	intra-typic	observed aa divergence: <4%	⇒	aa identity: >96%
	inter-typic (within species)	observed aa divergence: 6-30%	⇒	aa identity: 70-94%
	between species	observed aa divergence: 31-50%	⇒	aa identity: 50-69%
	between genera	observed aa divergence: 48-58%	⇒	aa identity: 42-52%