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:	***2015.020aM***	(to be completed by ICTV officers)

Short title: One new species in the genus *Rotavirus*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)					
	1 ☒	**2** ☒	**3** ☐	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☒

Author(s):

Mihalov-Kovács, E, Gellért, Á, Marton, S, Farkas, SL, Fehér, E, Oldal, M, Jakab, F, Martella, V, Bányai, K

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Mihalov-Kovács, Eszter (kovacsesztermail@gmail.com), Bányai, Krisztián (bkrota@hotmail.com)

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)	*Reoviridae* Study Group

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

Page 1/5:

List the ICTV study group(s) that have seen this proposal:

Add: "Reoviridae Study Group"
Response: This is added.

Page 2/5:

MODULE 2:
To create ____ new species within:

Add "1".
Response: This is added.

The authors added information about two representative isolates. Please reduce to a single representative isolate as requested.

Response: We reduced the number of representative strains.

Page 3/5:

With respect to the "Host species".
Please provide evidence for the claim that RVI may also be present in sea lions and cats. Have any sequence comparisons been done between the cat/sea lion/dog RV sequences?

Response: Phylogenetic trees are now included to show this evidence

Page 4/5:

MODULE 10:
Please describe and provide phylogenetic evidence that the 10 other gene segments (in addition to VP6) of the described canine RV strains are also distinct from known rotavirus species (A-H).

Response: Phylogenetic trees are added to this section.

Dear …

The Study Group Reoviridae has assessed your proposal in great detail, and has the following remarks/questions/comments:

Comment 1: The proposal would be significantly stronger if the proposed species I rotavirus would be isolated in cell culture. Has this been attempted? Isolation of the virus in culture would ascertain that all the described rotavirus gene segments are from a single virus, and that the virus is available for future studies or other researchers.
Response: Our colleague, Vito Martella, professor at University of Bari (co-author of the paper describing the novel RV in sheltered dogs), has attempted the cultivation of Italian RVI strains he identified during a survey of diarrheic dogs. These attempts remained unsuccessful (information kindly shared by Prof. Martella). However, this is not surprising given that only RVA strains can be isolated at relatively high success rate. In addition, a few RVC and RVH strains can be also cultivated but no strains belonging to other RV species are cultivatable by conventional methods.

Comment 2: Phylogenetic evidence should be presented for all 11 gene segments of the proposed species I rotavirus to prove that all the eleven gene segments are highly distinct from other established rotavirus species (A-D, F-H).
Response: The requested phylogenetic trees are now added to the proposal.

Comment 3: As there are no sequence data available for RVE, the only way to ascertain that the proposed species I rotavirus does not belong to the RVE species, is to compare the electropherotype of the proposed species I rotavirus with the electropherotype of RVE and other rotavirus species.
Response. Although attempts were made to electropherotype the genomic RNA of RVI, however, viral titer was very low. We encountered the same issue when trying to electropherotype the RNA extracts prepared from RVI positive stool samples of Italian and Turkish dogs. Thus, the virus seems to be shed at low quantity under natural circumstances. However, we think that low virus titer may be relevant regarding the pathogeneicity but should not prevent the classification of a novel rotavirus.

With the critical comment in mind we would like to point out that the electropherotypes of RVH strains and RVE strains have never been compared in the same polyacrylamide gel. This is particularly painful as both RVH and RVE seem to be common in pigs, even though RVE, at least in its originally described form, was not detected during the past 2 decades or so. Thus, the shortcoming that is criticized by the ICTV's study group is valid for RVH as well. Unfortunately, the question whether RVE declined over time in pig population or modern strains underwent extensive genetic changes that led to changes in their RNA profile remains open in the absence of adequate reference material for RVE.

Comment 4: As it is believed that the terminal sequences of each of the gene segments is crucial for packaging into novel rotavirus particles, the comparison of these gene ends between the proposed species I rotavirus and established rotavirus species should be made, in order to assess the theoretical possibilities of reassortment between the novel virus and rotavirus belonging to other species.

Response: The terminal sequences were determined and the GenBank records were updated with this information.

Comment 5: The proposed species I has been described in a rather small set of independent studies. Additional examples of its genetically stable circulation in nature would support the new species assignment.

Response: In collaborative studies we found evidence that RVI circulate in Italian and Turkish dogs (unpublished data). In addition, novel RV sequences in the feline and seal fecal virome have been reported from the USA (by Delwart et al.). We analyzed these sequences and concluded that they likely belong to the proposed new RV species, RVI (phylogenetic trees are added to the proposal).

Date first submitted to ICTV:	03/17/2015
Date of this revision (if different to above):	07/05/2015

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	***2015.020aM***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Rotavirus***
Subfamily:	***Sedoreovirinae***
Family:	***Reoviridae***
Order:	***Unassigned***

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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Rotavirus I	RVI/Dog-wt/HUN/KE135/2012/G1P1	RVI/Dog-wt/HUN/KE135/2012/G1P1 Seg5 KM369887 Seg8 KM369888 Seg7 KM369889 Seg10 KM369890 Seg11 KM369891 Seg1 KM369892 Seg2 KM369893 Seg3 KM369894 Seg4 KM369895 Seg6 KM369896 Seg9 KM369897

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 9

Virus discovery

Mihalov-Kovács et al. (2015) recently described the molecular detection of a tentatively new rotavirus species in dogs in Hungary [1].

We performed genomic dsRNA enrichment with KE135/2012 by differential LiCl precipitation; however, the enriched dsRNA remained invisible by PAGE and silver staining, because of the apparent low titer of the novel RV.

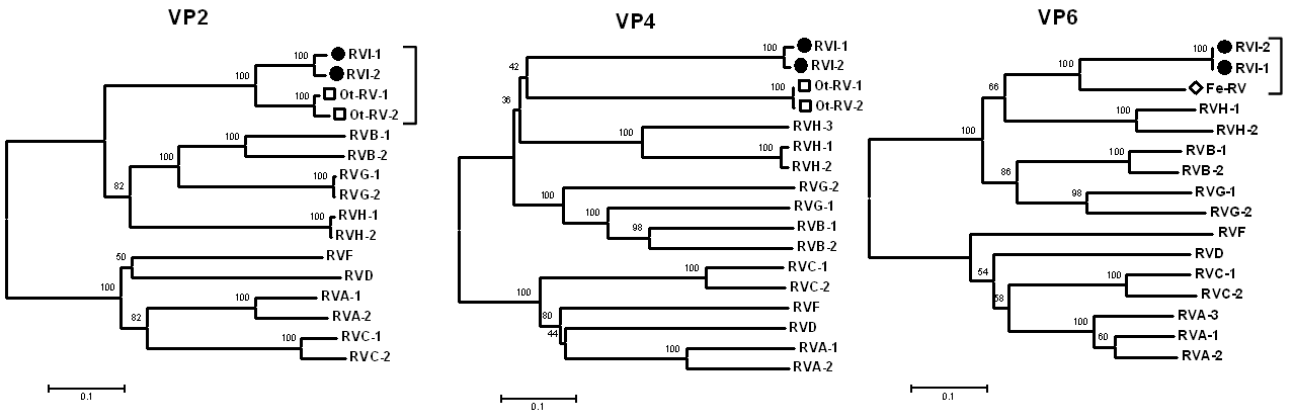
The VP6 aa sequence of the candidate RVI strain(s) shared the highest amino acid sequence identities with the RVH strains – 46%, and ranged between 13-38% with other RVs. This fact fulfills the species demarcation criteria of the RVs according to the suggestion of Matthijnssens et al., 2012 [2].

Phylogenetic analysis of the VP6 indicated the separation of the novel RVI strain(s) from established RV species. This is valid for the remaining 10 genes too.
The sequences of the two strains were submitted to GenBank on 20-AUG-2014; the data are now freely available.

Growth in cell cultures
The virus has not been isolated in cell culture. Attempts were made but failed.

Host species
We described these unusual RVs in the fecal specimen of two dogs (*Canis lupus familiaris*), and also seems to be evident that the tentative RVI was found in California sea lions (*Zalophus californianus*) and in domestic cat (*Felis silvestris catus*) in the recent years [3, 4].

Fig 1. Phylogenetic trees obtained for the partial sequences using unusual feline (Fe-RV) and otarine RV (Ot-RV-1 and Ot-RV-2) gene sequences. The alignments of the VP2, VP4 and VP6 proteins encompassed ~160, ~310, and ~70 aa long sequences. RVI-1 and RVI-2 represents two RVI strains, KE135/2012 and KE528/2012, respectively.



MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Mihalov-Kovács E, Gellért Á, Marton S, et al. (2015) Candidate New Rotavirus Species in Sheltered Dogs, Hungary. Emerg Infect Dis. doi: 10.3201/eid2104.141370

2. Matthijnssens J, Otto PH, Ciarlet M, et al. (2012) VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. Arch Virol 157:1177–1182. doi: 10.1007/s00705-012-1273-3

3. Li L, Shan T, Wang C, et al. (2011) The Fecal Viral Flora of California Sea Lions. J Virol 85:9909–9917. doi: 10.1128/JVI.05026-11

4. Ng TFF, Mesquita JR, Nascimento MSJ, et al. (2014) Feline fecal virome reveals novel and prevalent enteric viruses. Vet Microbiol 171:102–111. doi: 10.1016/j.vetmic.2014.04.005

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.

Table 1. Percentile amino acid sequence based identities between the novel canine RV strain, KE135/2012, and reference RVA-RVD and RVF-RVH strains.

	RVA	RVB	RVC	RVD	RVF	RVG	RVH
VP1	23	53	21	22	23	55	57
VP2	15	42	14	13	14	41	45
VP3	15	32	12	14	13	33	39
VP4	11	24	12	12	11	26	27
VP6	16	38	14	14	13	38	46
VP7	17	26	11	14	11	22	29
NSP1	<10	≤10	<10	<10	<10	<10	20-21
NSP2	15	42	16	15	14	41	42
NSP3	21	14-21	10-12	<10	12	18-20	15-16
NSP4	9-13	11	<10	11	10	10-11	14
NSP5	<10	25	<10	<10	<10	29	30

Fig. 2. Protein sequence based phylogenetic tree of the VP6 gene obtained by the neighbour-joining algorithm. Asterisks indicate >90% bootstrap values. The two Hungarian canine RV strains belonging to the proposed novel *Rotavirus I* clusters with RVH, RVG and RVB within a major clade referred to as Clade 2. RVA, RVC, RVD and RVF strains belong to Clade 1.

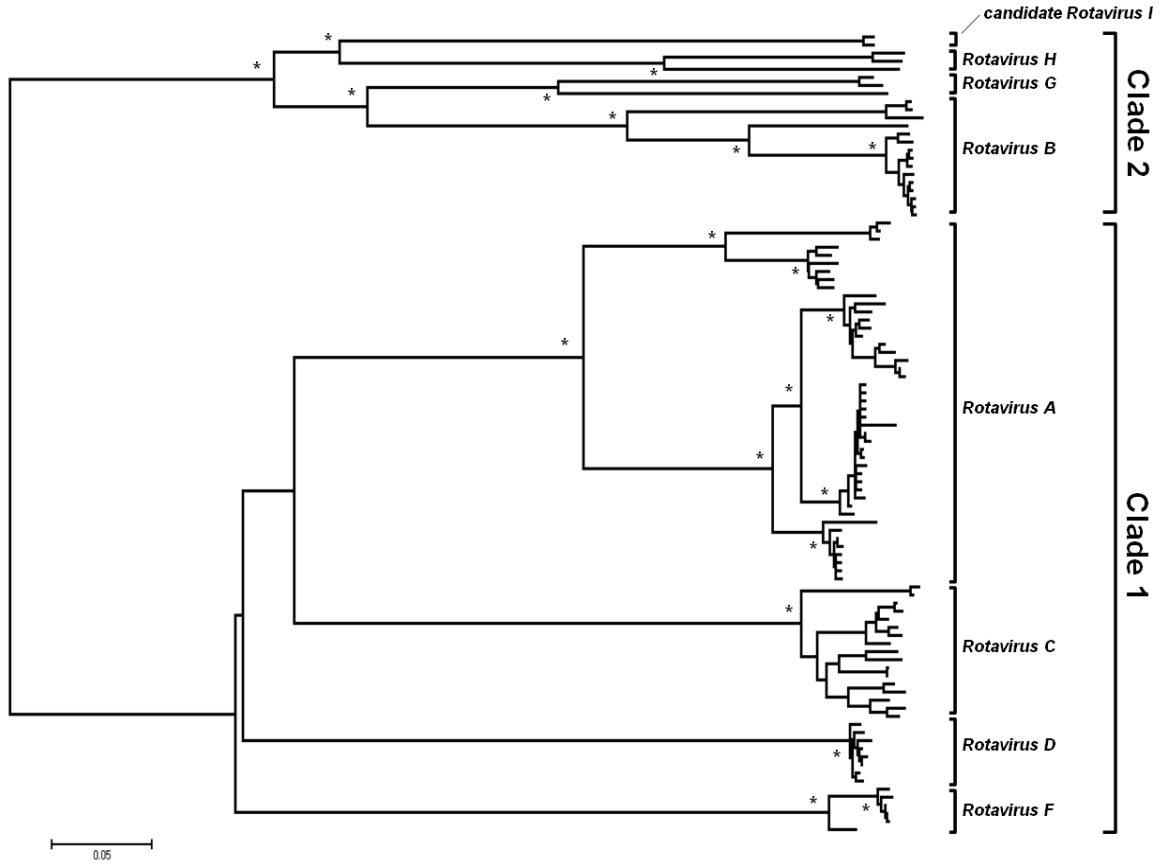
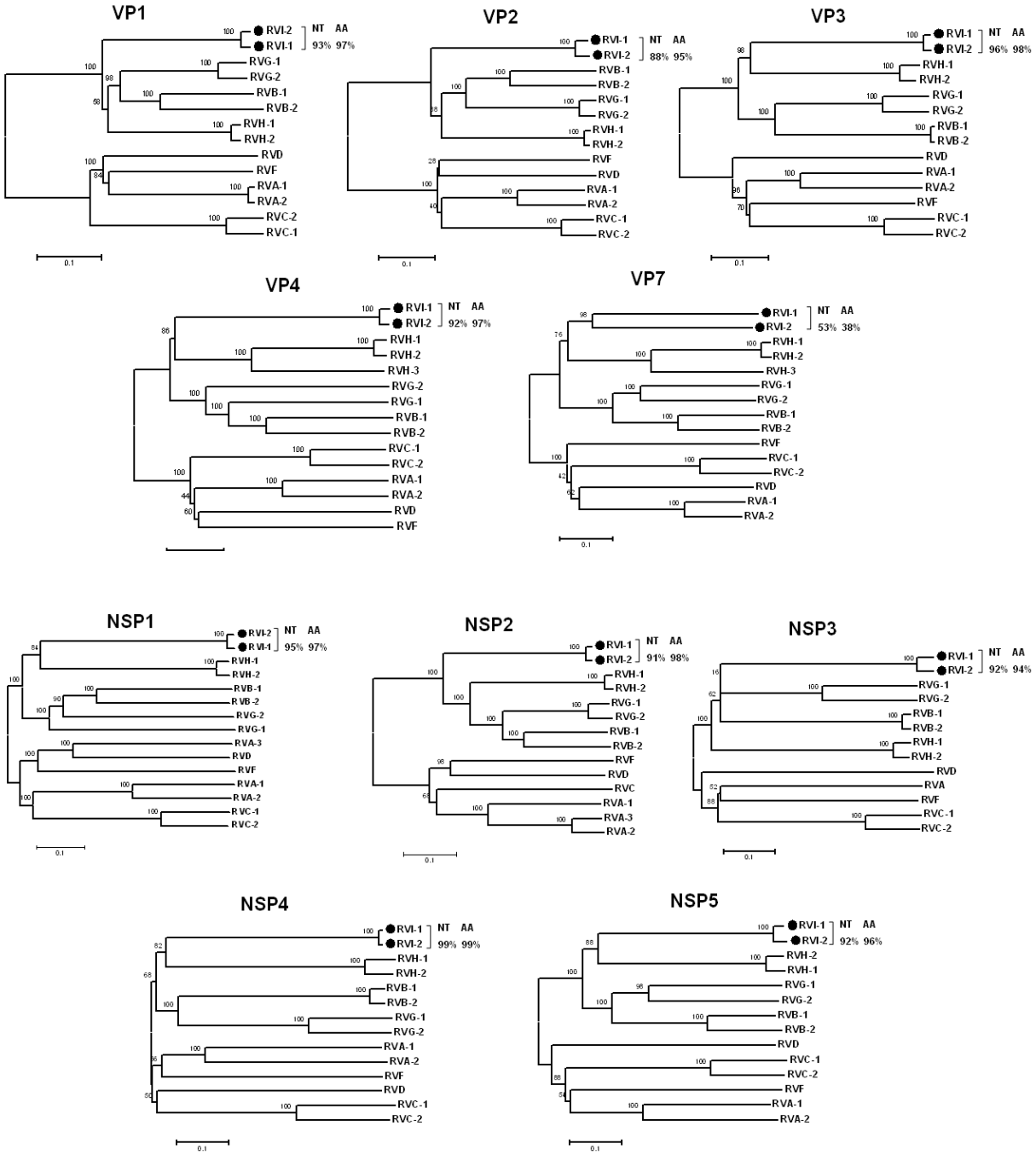


Fig 3. Phylogenetic trees obtained for the VP1 to VP4, VP7, NSP1 to NSP5 proteins with representative strains of RVA to RVH. RVI-1 and RVI-2 represents KE135/2012 and KE528/2012, respectively. Alignments were created using the BLOSUM62 algorithm as implemented at the Multalin website (http://multalin.toulouse.inra.fr/multalin/). Phylogenetic trees were prepared using the neighbor-joining method. Bootstrap values are shown at the branch nodes. Nucleotide and amino acid identities between KE135/2012 and KE528/2014 are show on the right.


VP1
RVI-2
RVI-1
NT AA
93% 97%
RVG-1
RVG-2
RVB-1
RVB-2
RVH-1
RVH-2
RVD
RVF
RVA-1
RVA-2
RVC-2
RVC-1
0.1
VP2
RVI-1
RVI-2
NT AA
88% 95%
RVB-1
RVB-2
RVG-1
RVG-2
RVH-1
RVH-2
RVF
RVD
RVA-1
RVA-2
RVC-1
RVC-2
0.1
VP3
RVI-1
RVI-2
NT AA
96% 98%
RVH-1
RVH-2
RVG-1
RVG-2
RVB-1
RVB-2
RVD
RVA-1
RVA-2
RVF
RVC-1
RVC-2
0.1
VP4
RVI-1
RVI-2
NT AA
92% 97%
RVH-1
RVH-2
RVH-3
RVG-2
RVG-1
RVB-1
RVB-2
RVC-1
RVC-2
RVA-1
RVA-2
RVD
RVF
VP7
RVI-1
RVI-2
NT AA
53% 38%
RVH-1
RVH-2
RVH-3
RVG-1
RVG-2
RVB-1
RVB-2
RVF
RVC-1
RVC-2
RVD
RVA-1
RVA-2
0.1
NSP1
RVI-2
RVI-1
NT AA
95% 97%
RVH-1
RVH-2
RVB-1
RVB-2
RVG-2
RVG-1
RVA-3
RVD
RVF
RVA-1
RVA-2
RVC-1
RVC-2
0.1
NSP2
RVI-1
RVI-2
NT AA
91% 98%
RVH-1
RVH-2
RVG-1
RVG-2
RVB-1
RVB-2
RVF
RVD
RVC
RVA-1
RVA-3
RVA-2
0.1
NSP3
RVI-1
RVI-2
NT AA
92% 94%
RVG-1
RVG-2
RVB-1
RVB-2
RVH-1
RVH-2
RVD
RVA
RVF
RVC-1
RVC-2
0.1
NSP4
RVI-1
RVI-2
NT AA
99% 99%
RVH-1
RVH-2
RVB-1
RVB-2
RVG-1
RVG-2
RVA-1
RVA-2
RVF
RVD
RVC-1
RVC-2
0.1
NSP5
RVI-1
RVI-2
NT AA
92% 96%
RVH-2
RVH-1
RVG-1
RVG-2
RVB-1
RVB-2
RVD
RVC-1
RVC-2
RVF
RVA-1
RVA-2
0.1