



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**

<b>Code assigned:</b>	<b>2014.002aV</b>	(to be completed by ICTV officers)			
<b>Short title:</b> Create 3 new rotavirus species ( <i>Rotavirus F</i> , <i>Rotavirus G</i> and <i>Rotavirus H</i> ) in the existing genus <i>Rotavirus</i> (e.g. 6 new species in the genus <i>Zetavirus</i> )					
<b>Modules attached</b> (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/> 6 <input type="checkbox"/>	2 <input checked="" type="checkbox"/> 7 <input type="checkbox"/>	3 <input type="checkbox"/> 8 <input type="checkbox"/>	4 <input type="checkbox"/> 9 <input checked="" type="checkbox"/>	5 <input type="checkbox"/>

**Author(s) with e-mail address(es) of the proposer:**

Jelle Matthijnsens ([jelle.matthijnsens@gmail.com](mailto:jelle.matthijnsens@gmail.com)), Reimar Johnne ([Reimar.Johnne@bfr.bund.de](mailto:Reimar.Johnne@bfr.bund.de)) and Ulrich Desselberger ([ud207@medschl.cam.ac.uk](mailto:ud207@medschl.cam.ac.uk))

**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-EC or Study Group comments and response of the proposer:**

These proposals were presented to Houssam Attoui, the chair of the *Reoviridae* Study Group. He suggested a minor amendment to the explanatory text, which has been made.

Futher comments from the EC:

1. Update the species definition on page 4. This was recently modified to: "A species is a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria."
2. Replace the abbreviations (RVA, group A, *rotavirus A*, etc.) in the various trees by the actual or proposed species names (*Rotavirus A*, etc.)
3. At the top of page 7, replace "RV species H (RVH)" by the proposed species name.

These amendments have been made.

Date first submitted to ICTV:

May, 2014

Date of this revision (if different to above):

31 July 2014

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	<b>2014.002aV</b>	(assigned by ICTV officers)
<b>To create 3 new species within:</b>		
Genus:	<i>Rotavirus</i>	Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “ <b>(new)</b> ” after its proposed name. • If no genus is specified, enter “ <b>unassigned</b> ” in the genus box.
Subfamily:	<i>Sedoreovirinae</i>	
Family:	<i>Reoviridae</i>	
Order:	-	
<b>Name of new species:</b>	<b>Representative isolate:</b>	<b>GenBank sequence accession number(s)</b>
<i>Rotavirus F</i>	Rotavirus F RVF/Chicken- wt/DEU/03V0568/2003/GXP[X]	[Seg1: JN596591, Seg2: JQ919995, Seg3: JQ919997, Seg4: JQ919996, Seg5: JQ919999, Seg6: HQ403603, Seg7: JQ920001, Seg8: JQ920000, Seg9: JQ919998, Seg10: JQ920003, Seg11: JQ920002]
<i>Rotavirus G</i>	Rotavirus G RVG/Chicken- wt/DEU/03V0567/2003/GXP[X]	[Seg1: JN596592, Seg2: JQ920004, Seg3: JQ920006, Seg4: JQ920005, Seg5: JQ920008, Seg6: HQ403604, Seg7: JQ920010, Seg8: JQ920009, Seg9: JQ920007, Seg10: JQ920011, Seg11: JQ920012]
<i>Rotavirus H</i>	Rotavirus H RVX/Human-tc/CHN/NADRV- J19/1997/GXP[X]	[Seg1: DQ113897, Seg2: DQ113898, Seg3: DQ113899, Seg4: DQ113900, Seg5: DQ113901, Seg6: DQ113902, Seg7: DQ113903, Seg8: DQ113904, Seg9: DQ113905, Seg10: DQ113906, Seg11: DQ113907]

## Reasons to justify the creation and assignment of the new species:

Rotaviruses (RVs) are members of the *Reoviridae* family, causing severe diarrheal illness in animals of many mammalian and avian species, and in humans [1]. The RV infectious particle is composed of three concentric protein layers. Viral protein (VP) 7 and VP4 are the components of the outer layer (outer capsid) and carry the neutralization-specific epitopes. The middle layer (inner capsid) is composed of VP6 and surrounds the core (inner layer). The latter is composed of VP2 (as the scaffolding protein), the enzymatic replication complexes (VP1 and VP3), and the 11 segments of the dsRNA genome [2, 3]. The RV genome also encodes 5 or 6 non-structural proteins (NSP), with varying functions during viral replication and morphogenesis [2, 4]. RVs have been differentiated into species using indirect immunofluorescence techniques, complemented by RNA fingerprinting [5, 6]. Antisera used in indirect immunofluorescence methods were mainly directed against VP6 [7]. Five serological species (*Rotavirus A* through *Rotavirus E*), and two unassigned groups (*Rotavirus F* and *Rotavirus G*) are currently recognized according to the International Committee on Taxonomy of Viruses (ICTV) [1]. Unfortunately, to date no sequence data are available for *Rotavirus E*. Attempts to recover the original strain are ongoing, but it is rather unlikely that this only currently known isolate will be recovered for sequence analyses. The “RV species” are commonly referred to as “RV groups” in the scientific literature. The RVA, RVB and RVC groups are known to infect humans and animals, whereas the RVD, RVE, RVF and RVG groups only infect animals [2, 8]. Recently a new RV was discovered infecting adults, which did not belong to any of the established RV groups. This new virus was named “New adult diarrhea virus” or ADRV-N [9, 10].

Traditionally, viral classification has been based on clinical, morphological and serological characteristics of different virus strains (in this temporal order). With the rapidly increasing use of molecular techniques around the world which very often have become the ‘gold standard’ of diagnosis, sequence-based classification is complementing the more traditional classification methods. Construction of pairwise sequence identity profiles has been used to differentiate genera into virus species [11-13], or to define genotypes inside the species of viruses causing acute gastroenteritis [12, 14, 15].

In a recent study, VP6 sequence data for RVA, RVB, RVC, RVD, RVF, RVG and ADRV-N were retrieved from databases and exploited to develop a sequence-based classification strategy to distinguish viral species within the genus *Rotavirus* [16]. This paper proposed a 53% amino acid cut-off value of the RV protein VP6 to distinguish different RV species, recognizing 4 out of 5 RV species (corresponding to RVA-RVD) recognized by the ICTV (no sequence data for RVE are available), the 2 tentative RV species (RVF, RVG) and a novel species (RVH, formerly known as ADRV-N). In a later study it was shown that the remaining 10 gene segments of the RV strains belonging to these novel species also clustered in distinct phylogenetic clusters, further confirming their classification as distinct species [17].

Further material in support of this proposal is presented in Appendix, Module 9.

## MODULE 9: **APPENDIX**: supporting material

Two recent papers have led to this proposal [16, 17]. The main findings from these papers, including figures, have been added here:

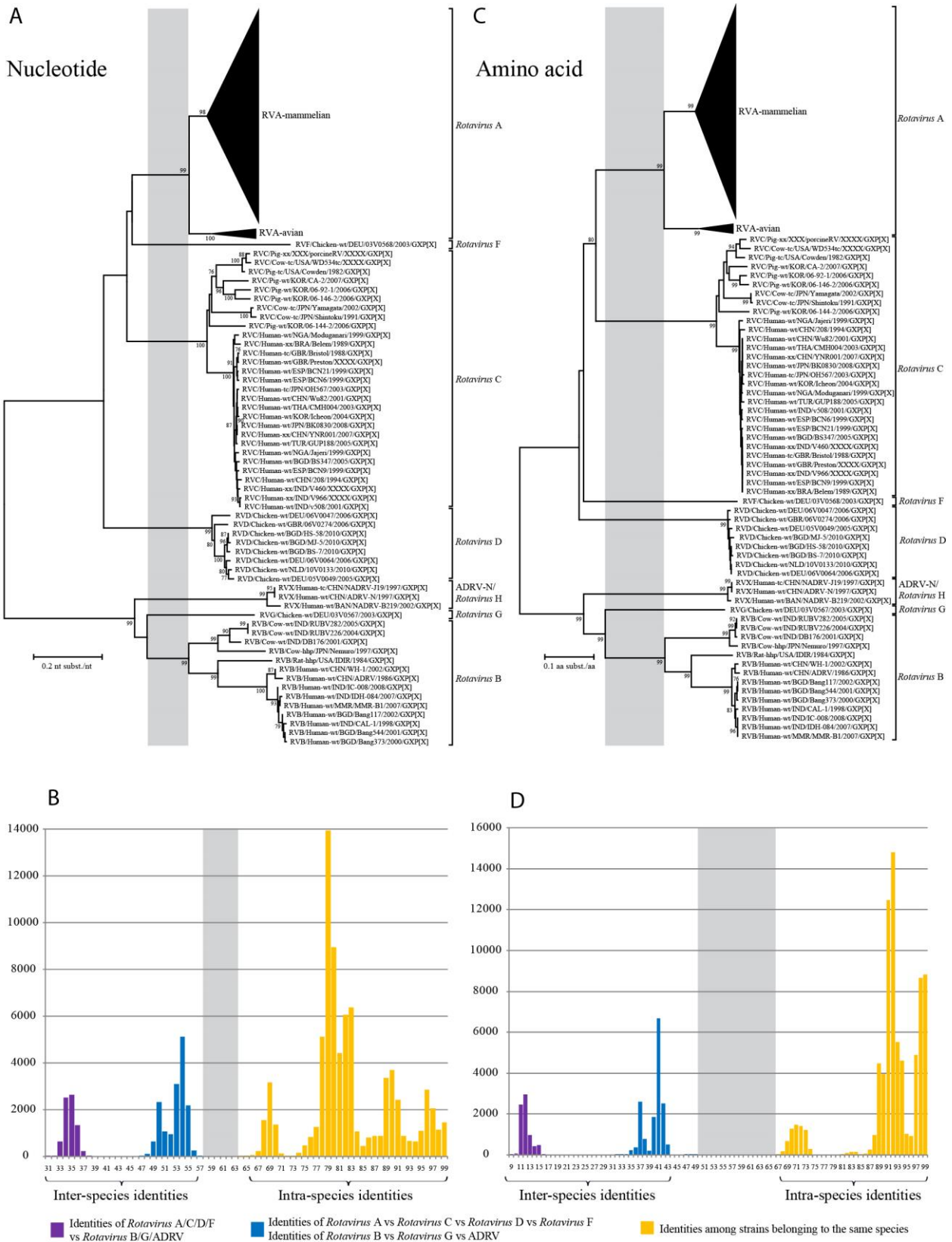
Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M, Johne R. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. Arch Virol. 2012 Jun;157(6):1177-82.

A total of 393 RVA, 14 RVB, 30 RVC, 8 RVD, 1 RVF, 1 RVG and 3 ADRV-N VP6 sequences were retrieved from GenBank. These sequences were aligned and the ORF regions were identified. The few available flanking non-coding regions were not considered in further calculations, and phylogenetic trees at the nt and aa levels were constructed (Fig 1A and C). Phylogenetic and molecular evolutionary analyses were conducted both at the nucleotide (nt) and amino acid (aa) level using the MEGA version 5 software [18]. Genetic distances were calculated using the Poisson correction parameter at the amino acid level and the Kimura-2 correction parameter at the nucleotide level. The dendrograms were constructed using the neighbor-joining method. To obtain suitable cut-off values for sequence-based classification, the percentage nt and aa identities between the complete open reading frames (ORFs) of more than 400 VP6 were calculated using the pairwise distances program of the MEGA version 5 software [18]. The use of “pairwise identity frequency graphs” for the classification of viruses, has been recommended by the ICTV where appropriate [19], and has been used for RVA classification [14]. The pairwise identity frequency graphs were constructed by plotting all the calculated pairwise identities in a graph with the percentage identities in the abscissa (X-axis) and the frequency of each of the calculated pairwise identities in the ordinate (Y-axis).

Overall, the nt- and aa-based phylogenetic trees could be subdivided into two major clusters, one containing RVA, RVC, RVF and RVD, and the other RVB, RVG and ADRV-N. Furthermore, at both the nt and aa levels, each individual RV species formed a clear monophyletic branch (Figs 1A and 1C). In addition, pairwise identities were calculated in order to construct pairwise nt and aa identity graphs (Fig 1B and D). The analyses indicated that the investigated RV species were clearly separated and formed well resolved peaks. Identities between RV strains not clustering in the same major phylogenetic branch (RVA, RVC, RVF, RVD, or RVB, RVG, ADRV-N) ranged between 31-38% at the nt level and 9-16% at the aa level, as indicated in purple in Figs 1B and 1D, and Tables 1A and 1B. Identities between strains belonging to the same large phylogenetic branch, but in a different RV species, ranged from 47-57% at the nt level and 32-49% at the aa level, as indicated in blue in Figs 1B and 1D and Tables 1A and 1B. RV strains belonging to the same species showed identities ranging from 65-100% at the nt level and 67-100% at the aa level, as indicated in yellow in Figs 1B and 1D, and Tables 1A. The gray shaded areas in Figs 1B and 1D indicate the ranges of possible nt (58-64%) or aa (50-66%) cut-off values which are considered as suitable for distinguishing RV species.

The ICTV defines a virus species as: “*A species is a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria*”. In order to define a new species, a number of different demarcation criteria must be met. In recent years, phylogenetic analyses and pairwise identity graphs have become increasingly dominant demarcation criteria for virus species definition [19]. For RVs in particular, an important demarcation criterion to distinguish species is their “inability to exchange genetic material by genome reassortment during dual infections, thereby producing viable progeny virus strains” [1]. This inability to reassort cannot be easily tested, especially, since most RV species cannot be adapted to cell culture, to perform in vitro reassortment experiments. Therefore a number of other techniques have been used to distinguish RV species. RVs have historically been differentiated

into species using indirect immunofluorescence techniques, complemented with RNA fingerprinting [5, 6]. Antisera used in indirect immunofluorescence methods were mainly directed against VP6, forming the inner capsid of the RV particle [7].



**Figure 1:** Phylogenetic dendrograms of RV VP6 at the nucleotide level (panel A) and the amino acid level (panel C). Bootstrap values (500 replicates) above 70 are shown. Mammalian and avian RVA strains are replaced by triangles, in which the height of the triangle represents the number of sequences, and the width, the genetic diversity inside that cluster. Panels B and D show the respective identity frequency graphs. The gray shaded areas in panel B and D represent the range in which a suitable cutoff value can be chosen, to distinguish the currently established RV species. The gray shaded area in panels A and C indicate how the strains in the phylogenetic trees would be divided into species.

The VP6 based pairwise identity frequency analysis presented here indicates that the different established RV species can be clearly separated by sequence identity cut-off values at the nt and aa levels. At the nt level a range of cut-off values between 58% and 64% would be suitable, whereas at the aa level, a cut-off value between 50% and 66% would be adequate. Although both, the nt and the aa level appear to be appropriate to determine a suitable cut-off value, the resolution of the different peaks in the pairwise identity graphs is more widely spaced at the aa level, and therefore we propose to use an aa-based cut-off value to distinguish among different RV species. As it can be expected that the currently known diversity inside each of the distinct RV species will slowly expand due to the accumulation of point mutations over time, we consider it appropriate to use a cut-off value at the lower end of the suitable range (50-66%). Therefore, we propose a 53% aa cut-off value to differentiate distinctive RV species.

**Table 1:** Pairwise identity ranges of the VP6 genes and proteins of RVs. Panels A and B: Pairwise identity ranges between different RV groups (corresponding to species) at the nucleotide and amino acid levels, respectively.

<b>Panel A</b>							
<b>NT</b>	RVA	RVB	RVC	RVD	RVF	RVG	RVH
RVA	65-100						
RVB	31-37	65-100					
RVC	49-56	33-38	78-100				
RVD	47-52	32-35	47-51	90-99			
RVF	47-51	31-35	47-49	48-49			
RVG	33-37	55-57	34-37	35-36	33		
RVH	32-36	49-51	34-37	35-37	33	51-52	94-100
<b>Panel B</b>							
<b>AA</b>	RVA	RVB	RVC	RVD	RVF	RVG	RVH
RVA	67-100						
RVB	10-13	69-100					
RVC	38-43	12-16	86-100				
RVD	34-38	11-12	34-36	98-100			
RVF	35-38	12-13	32-34	36-37			
RVG	13-15	47-49	13-14	13-14	14		
RVH	12-15	36-38	14-15	15	9-10	41	98-100

Based on the results of the current analyses, the ADRV-N strains (represented by RVX/Human-wt/CHN/ADRV-N/1997/GXP[X], RVX/Human-tc/CHN/NADRV-J19/1997/GXP[X] and RVX/Human-wt/BAN/NADRV-B219/2002/GXP[X]) do not cluster together with any available sequences of the established RV species (RVA-RVD, RVF and RVG). The highest percentage of aa sequence identity is 41% by comparison to RVG. Therefore, by application of the 53% aa cut off value for differentiation of RV species, these strains have to be

classified into a new RV species. According to the practises used before, we propose to designate this novel species *Rotavirus H*.

Kindler E, Trojnar E, Heckel G, Otto PH, Johne R. Analysis of rotavirus species diversity and evolution including the newly determined full-length genome sequences of rotavirus F and G. *Infect Genet Evol.* 2013 Mar;14:58-67.

The first whole genomes of a RVF (strain 03V0568) and an RVG (strain 03V0567, both originating from chicken; [20]) were sequenced. Initial amplification of the genome segments was done by FLAC, a sequence-independent method for the amplification of full-length cDNA from double-stranded RNA templates [21-23]. As in most cases the complete genome segments could not be amplified by FLAC, primers with binding sites in conserved regions of avian and mammalian RV sequences were constructed; additional primer sequences were deduced after determination of partial genome segment sequences obtained by FLAC. The products were either sequenced directly or cloned and subsequently sequenced. The complete sequences of the genome segments were assembled from the sequence fragments using the SeqBuilder module of the DNASTAR software package (Lasergene, Madison, USA).

Sequence analyses of the generated genomes were done using 20 RVA strains, 6 RVB strains, 6 RVC strains, one RVD strain and two RVH strains retrieved from GenBank. These strains were selected for the availability of complete genomes (RVB-D, RVH) or a selection of complete genomes largely representing the genetic variability (RVA). Sequence alignments and calculations of sequence identities were performed using the MegAlign module of the DNASTAR software package (Lasergene), with the CLUSTAL W method and the IUB (nucleotides) or Gonnet 250 (amino acids) residue weight tables [24]. For phylogenetic analyses, the sequences were aligned for each segment at the amino acid level using the tool Muscle online available from the European Bioinformatics Institute and manually revised using the program Geneious 5.4.6. Phylogenetic relationships for each segment and for the concatenated genome sequences were inferred by Bayesian algorithms (BI) implemented in MrBayes 3.1.2 [25] and by Maximum Likelihood algorithms (ML) using the online tool PhyML [26]. Bayesian analyses were run at minimum for 1 million generations (at maximum for 10 million generations for the analysis of concatenated sequences), sampled every 1000th generation using one cold and three heated chains. The first 25% of the samples were discarded as burn-in and convergence was determined by examining the Potential Scale Reduction Factor (PSRF; around 1), the split frequencies (below 0.01) and by comparing the two runs using the program Tracer 1.5. The ML analyses were run with the default values suggested by PhyML and using the above mentioned substitution models. All trees were visualized in MEGA. RV strain designation, host species and GenBank accession numbers for all the sequences included in this phylogenetic study are directly indicated in the corresponding trees.

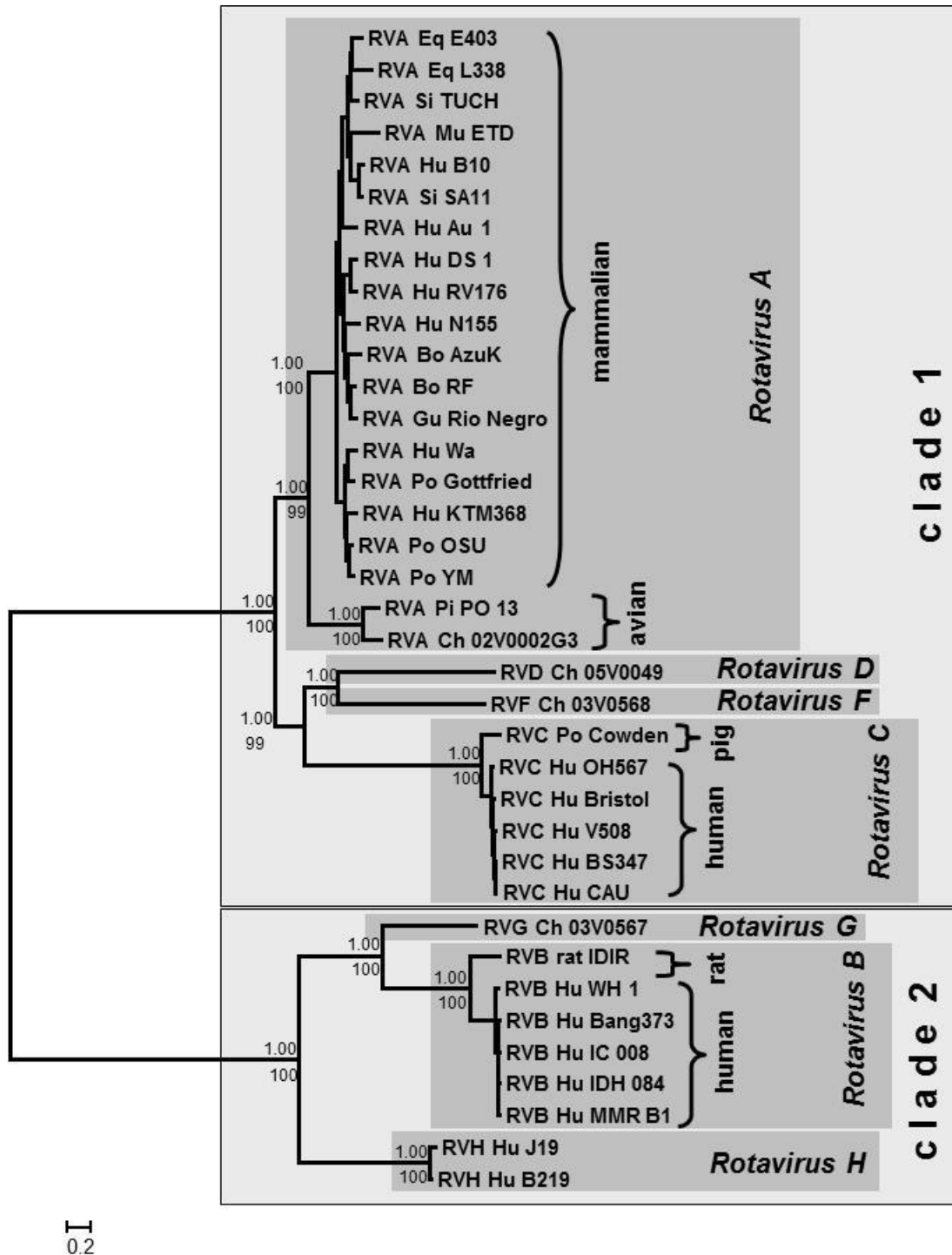
Nucleotide sequence identity of the segments derived from RVF and RVG compared to the other RV strains included in the analysis ranged between 29.8% and 65.9%. The amino acid sequences deduced from RVF and RVG genome segments have identities in a range between 5.2% and 59.1% compared to those of other RV species.

Phylogenetic analyses based on BI and ML algorithms congruently confirmed the assignment of RV sequences into seven well-defined clusters, thereby justifying the classification of *Rotavirus F*, *Rotavirus G* and *Rotavirus H* as separate species. Particularly, the analyses based on structural protein-encoding segments identified two highly supported major clades, consisting of RV A/C/D/F (clade 1) and RV B/G/H (clade 2). While phylogenetic trees based on non-structural protein genes NSP2 and NSP5 also showed a similar branching, trees based on the remaining segments encoding NSP1, NSP3 and NSP4 did not resolve these deeper relationships, most

possibly due to high sequence variability resulting in unsupported nodes and unresolved topology at the base of the trees (Supplementary data). However, the separation into the two well-defined clades is also supported by a genomic phylogenetic tree based on the analysis of the concatenated sequences of all eleven RV genome segments (Fig. 2). Even though this combined analysis evens differences in similarity patterns at particular loci potentially impacted by reassortment and recombination events, the majority of the genome sequence clearly shows the overall long-term independent evolution of the RV A/C/D/F and B/G/H clades.

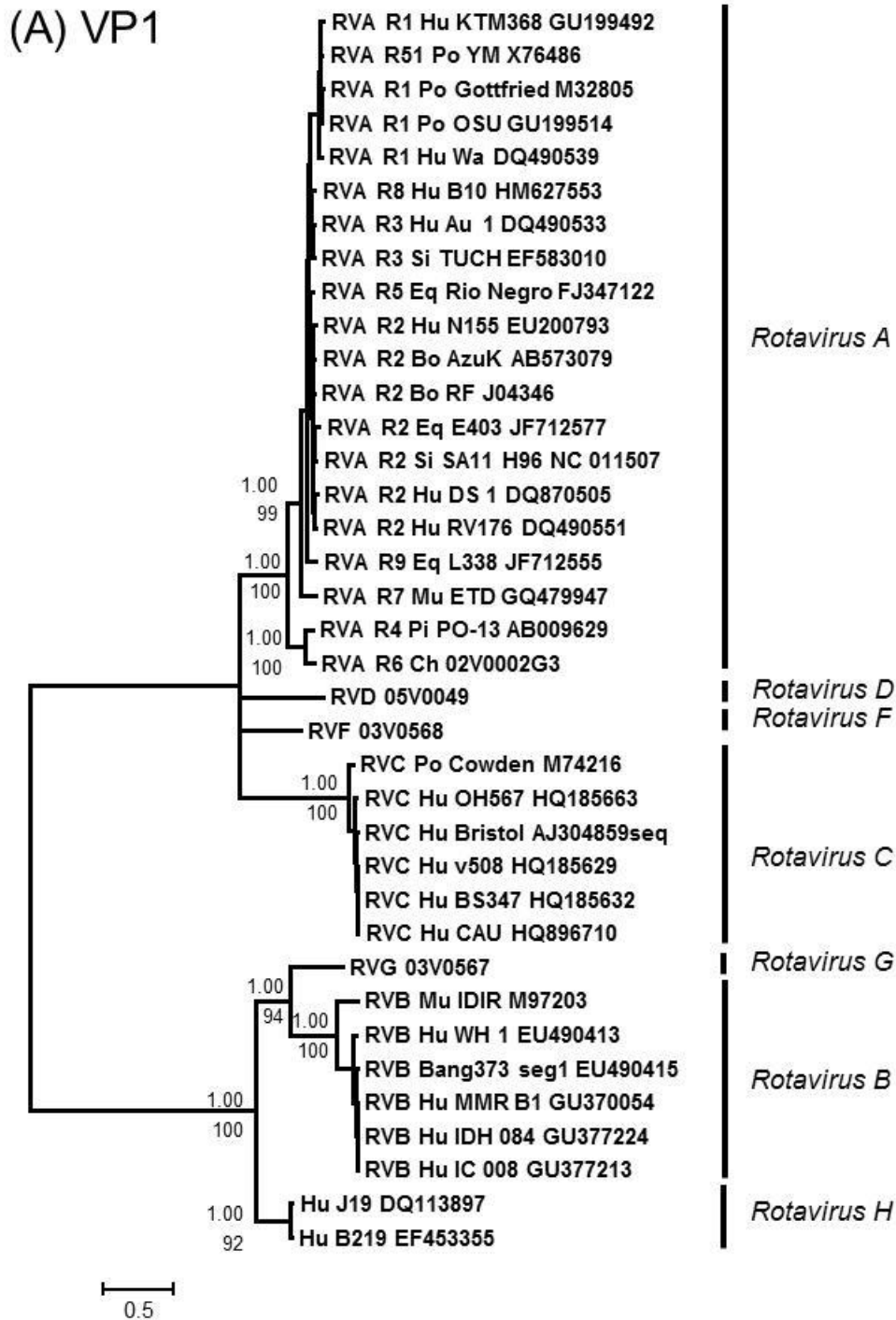
In the past, the assessment of RV species diversity was mainly based on results from studies on antibody cross-reactivity [2]. Recently, the availability of partial genome sequences led to a proposed classification system based on the VP6-encoding genome segment [16]. However, an independent development of distinct genome segments, e.g. through reassortment events, cannot be excluded. Therefore, the availability of a complete genome sequence is a prerequisite for an exact classification of a RV and for the reconstruction of the evolutionary history of RV species. The comparison of the complete genome sequences of RVF and RVG with genomes of other RV species generally revealed only low sequence identities and a separate branching in phylogenetic trees for all genome segments. Therefore, the presented data indicate that the assignment of separate species for RVF and RVG can be confirmed. Also, the grouping of the newly designated *Rotavirus H* as a separate species could be confirmed. Unfortunately, no sequences at all are available so far for RVE, therefore, every analysis of RV species diversification is still incomplete. There should be increased efforts to identify RVE strains in order to prove the existence and to complete the analysis of the species *Rotavirus E*.



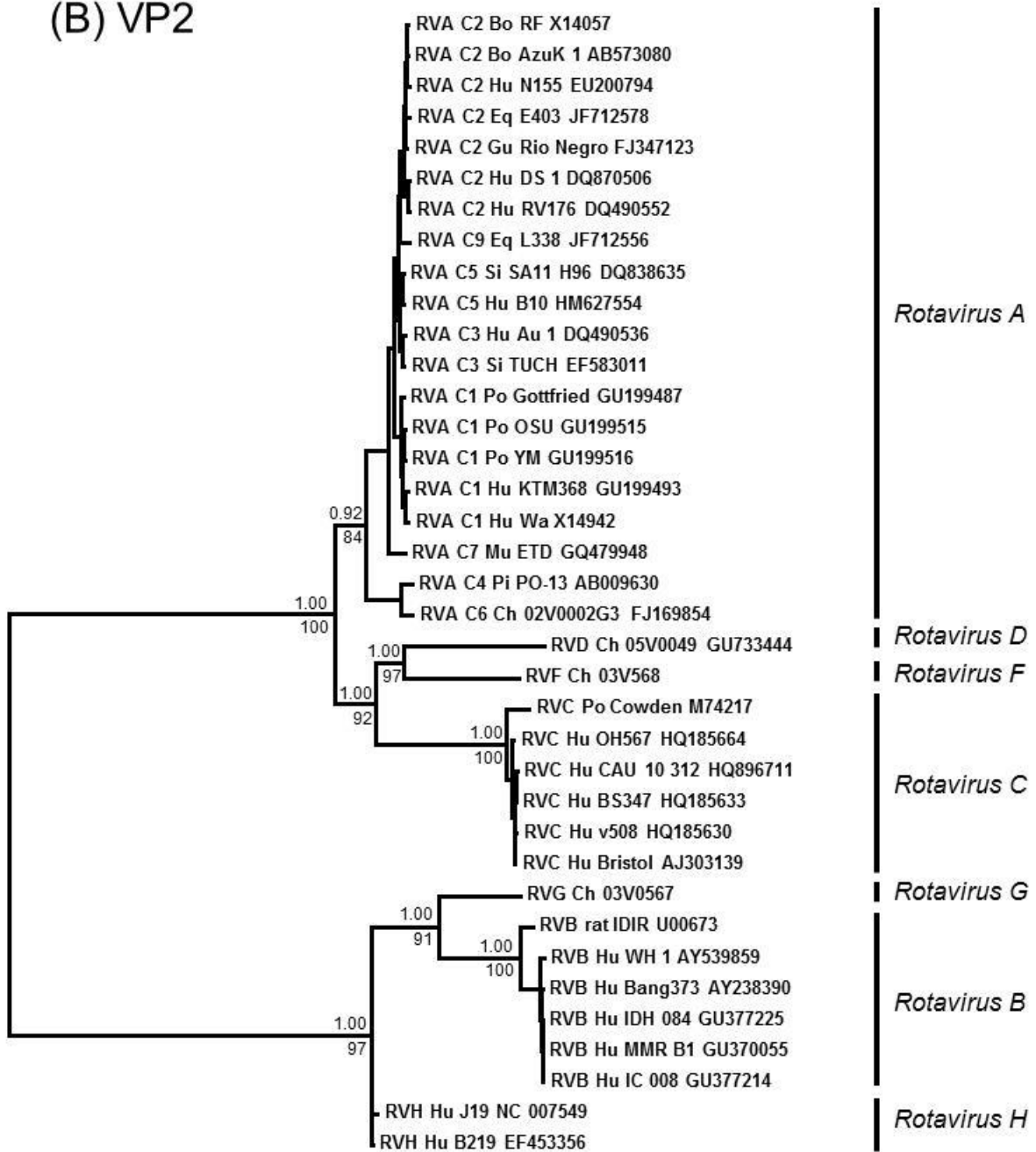


**Figure 2** Bayesian reconstruction of phylogenetic relationship of RV species based on their whole genome sequences. The genomic tree was created using the sequences of all 11 genome segments of the RV strains in concatenated analyses with Bayesian algorithms and by Maximum Likelihood (ML) algorithms (see the Methods Section for details). RV species (RVA-RVH), host abbreviation (Eq – equine, Si – simian, Mu – murine, Bo – bovine, Gu – guanaco, Po – porcine, Pi – pigeon, Ch – chicken, Rat – rat) and strain designations are indicated at the end of the branches. Grouping of the RVs into the RV species (RV A-H), into the two major clades and according to hosts is indicated. Posterior probabilities for Bayesian inference are indicated above the major branches and support values for ML analyses below the branches, respectively. \* indicates a different topology based on ML algorithms, ns refers to posterior probabilities < 0.70 and support values < 70%.

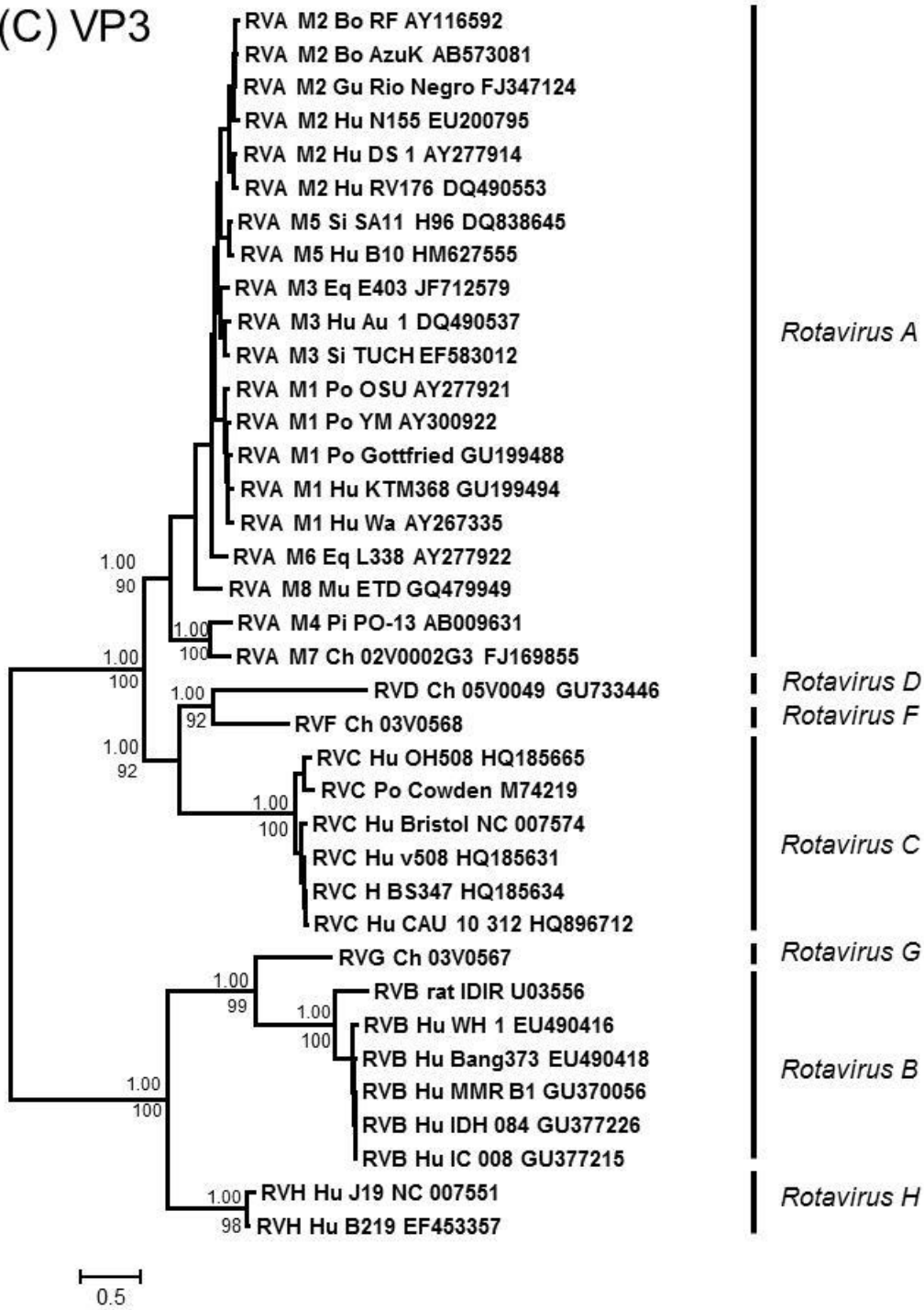
**Supplementary data:** Phylogenetic relationship of RV species based on the genome segments encoding (A) VP1, (B) VP2, (C) VP3, (D) VP4, (E) VP6, (F) VP7, (G) NSP1, (H) NSP2, (I) NSP3, (K) NSP4, (L) NSP5. The RV species (RVA – RVH), genotype (for RVA strains), host abbreviation (Eq – equine, Si – simian, Mu – murine, Bo – bovine, Gu – guanaco, Po – porcine, Pi – pigeon, Ch – chicken, Rat – rat), strain designation and GenBank accession numbers are indicated for each strain at the end of the branches. The trees for VP3 (S4C) and NSP1 (S4G) are also shown in Fig. 2. Phylogenetic relationships were inferred by Bayesian algorithms and by Maximum Likelihood (ML) algorithms (see the Methods Section for details). Posterior probabilities for Bayesian inference are indicated above the major branches and support values for ML analyses below the branches, respectively. \* indicates a different topology based on ML algorithms, ns refers to posterior probabilities < 0.70 and support values < 70%.



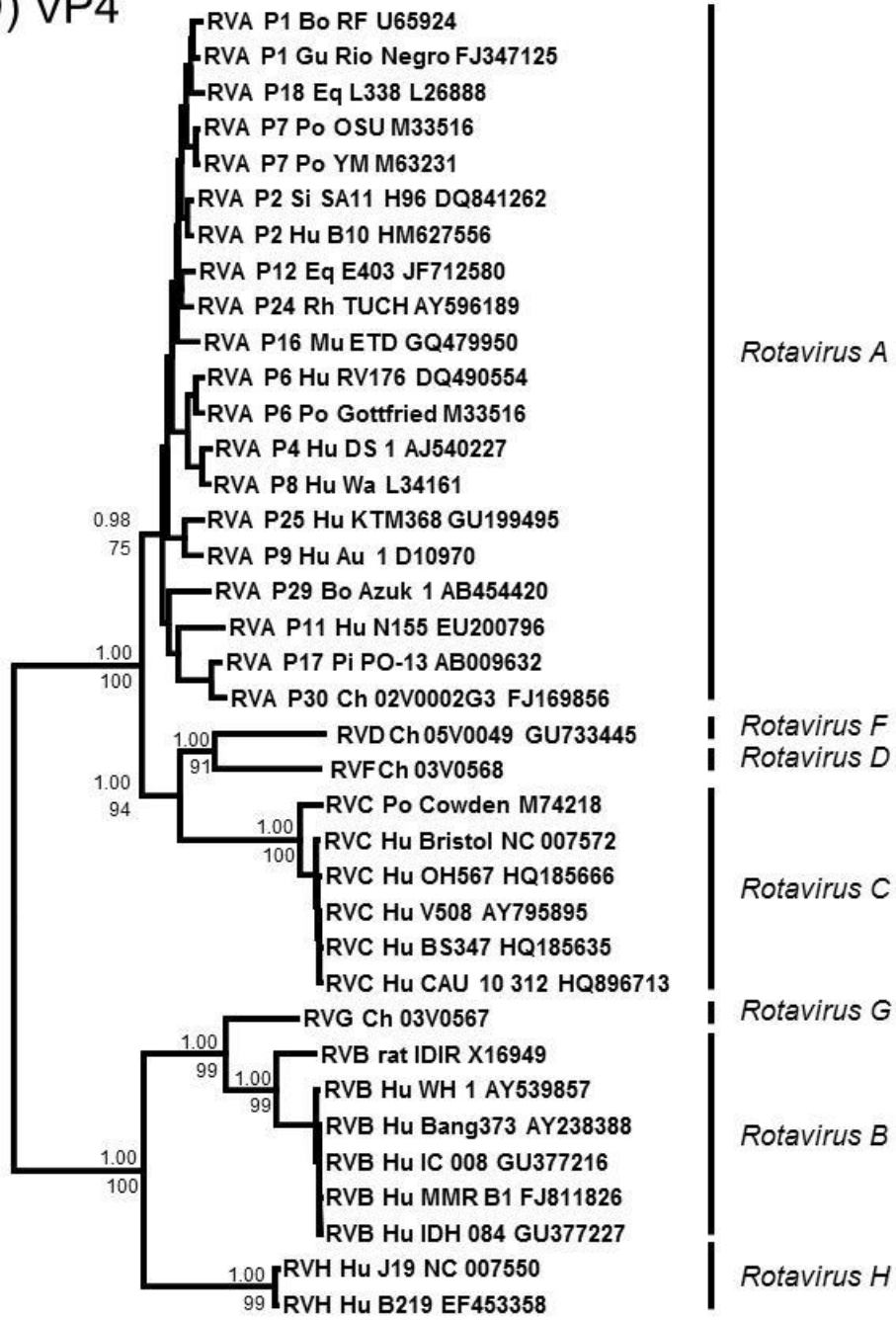
(B) VP2



(C) VP3

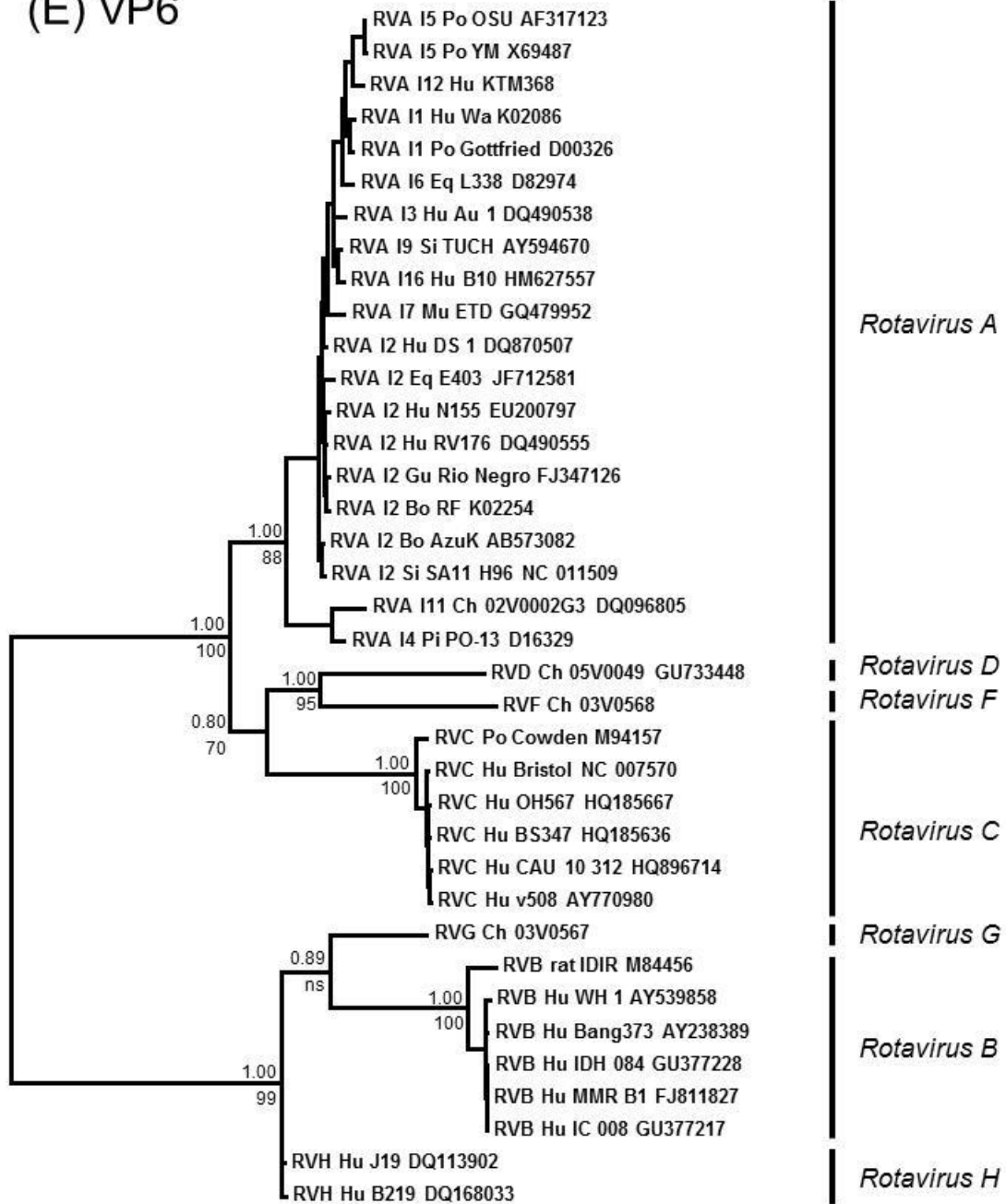


(D) VP4



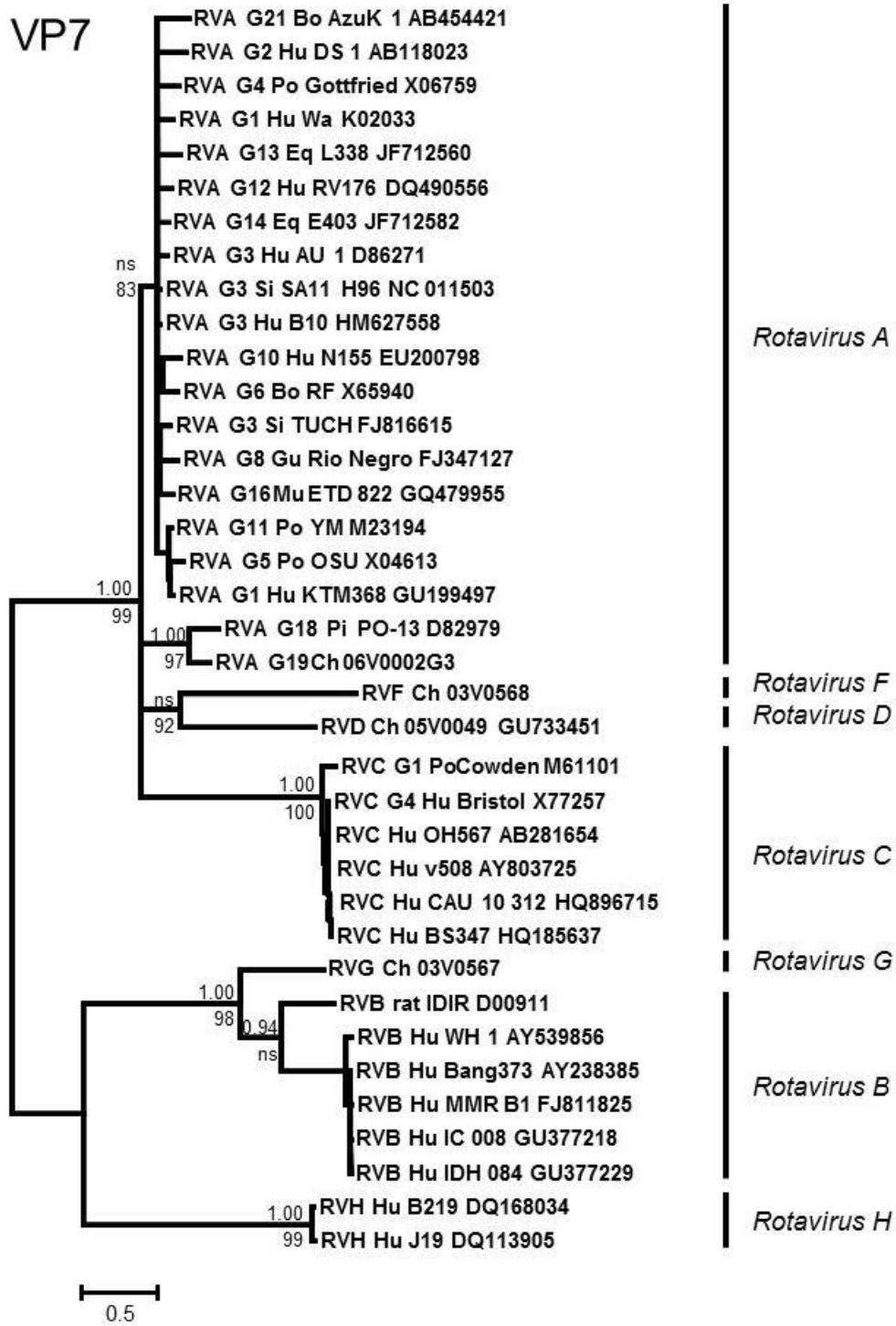
0.2

(E) VP6

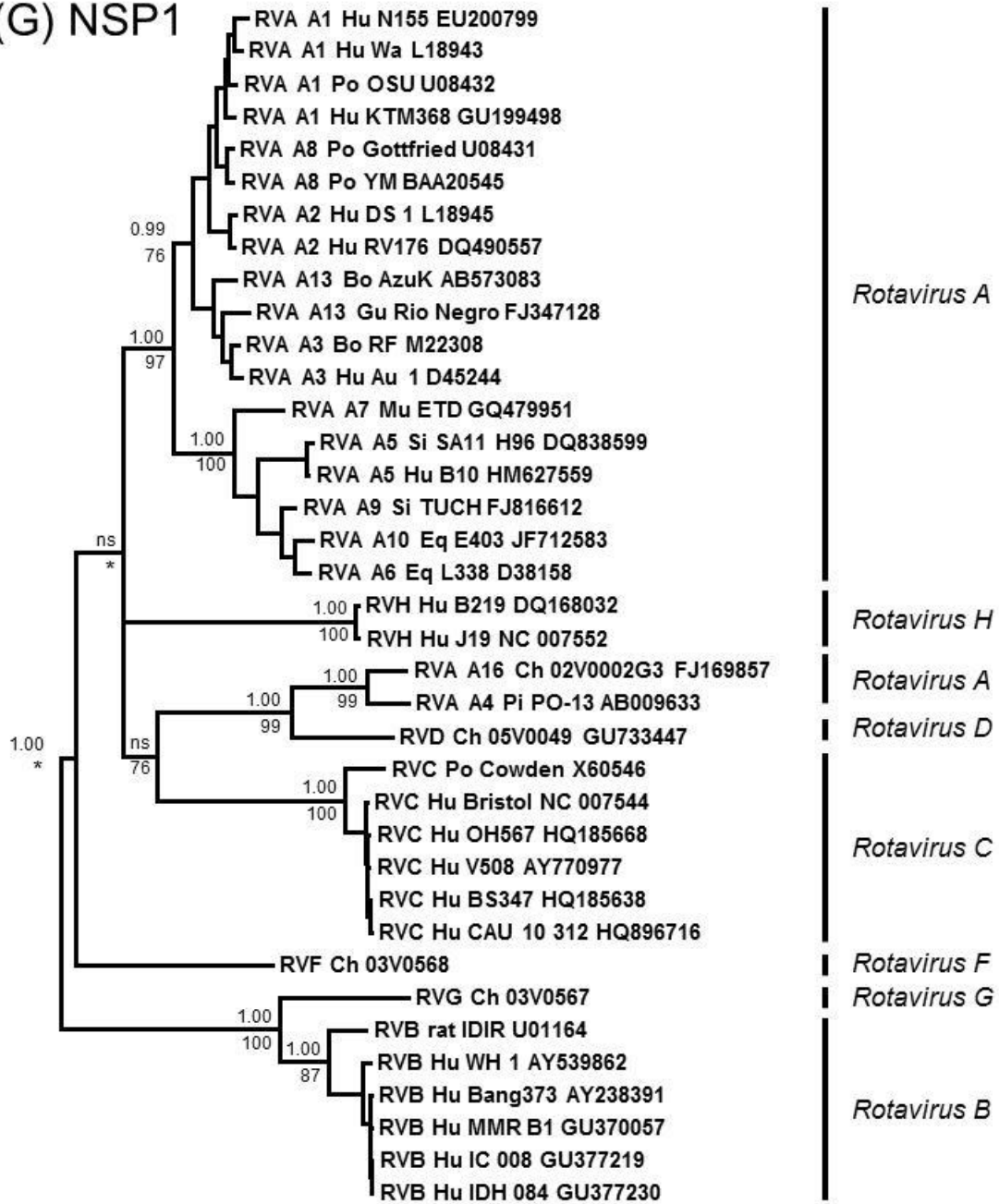


0.5

(F) VP7

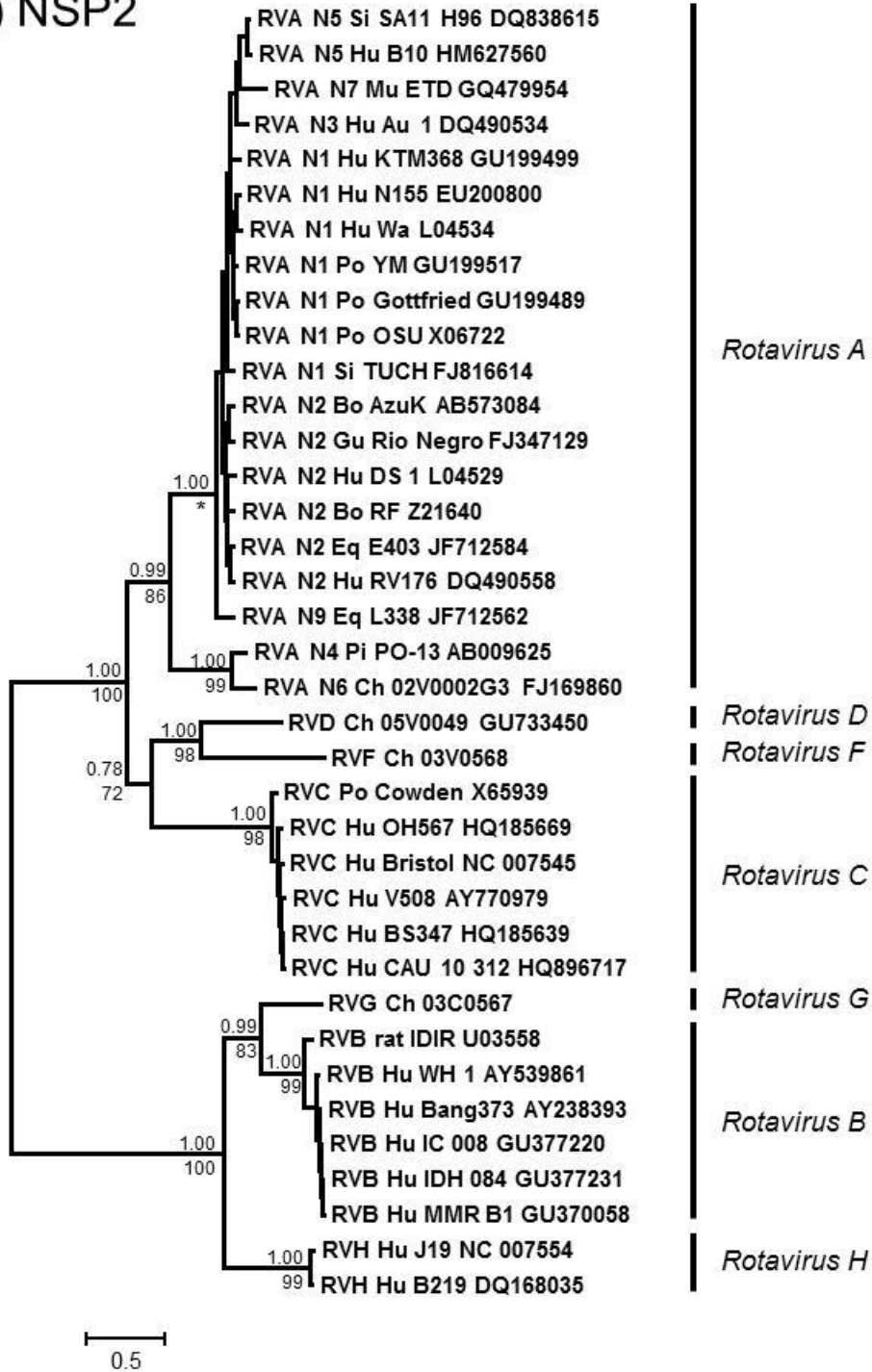


(G) NSP1

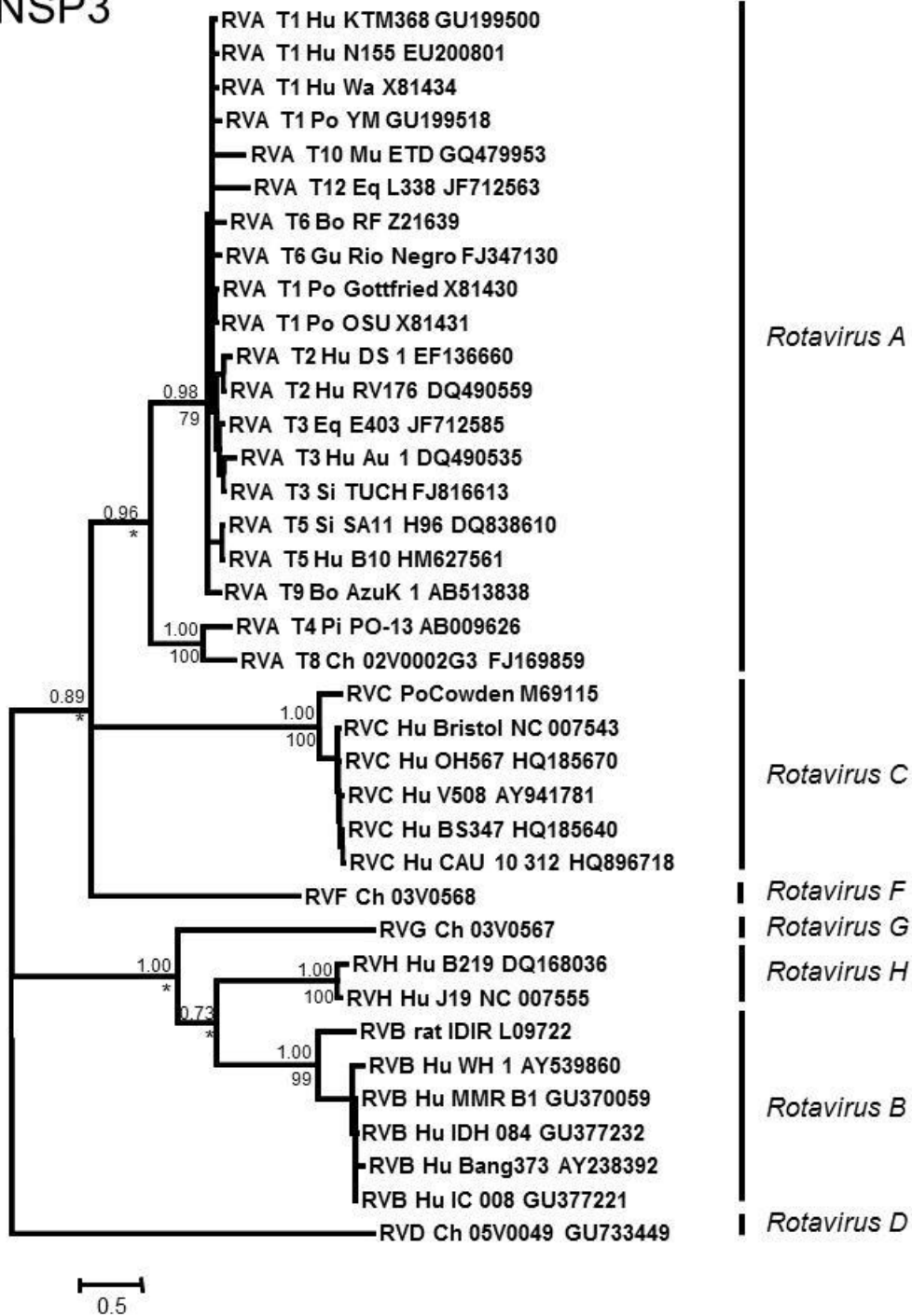




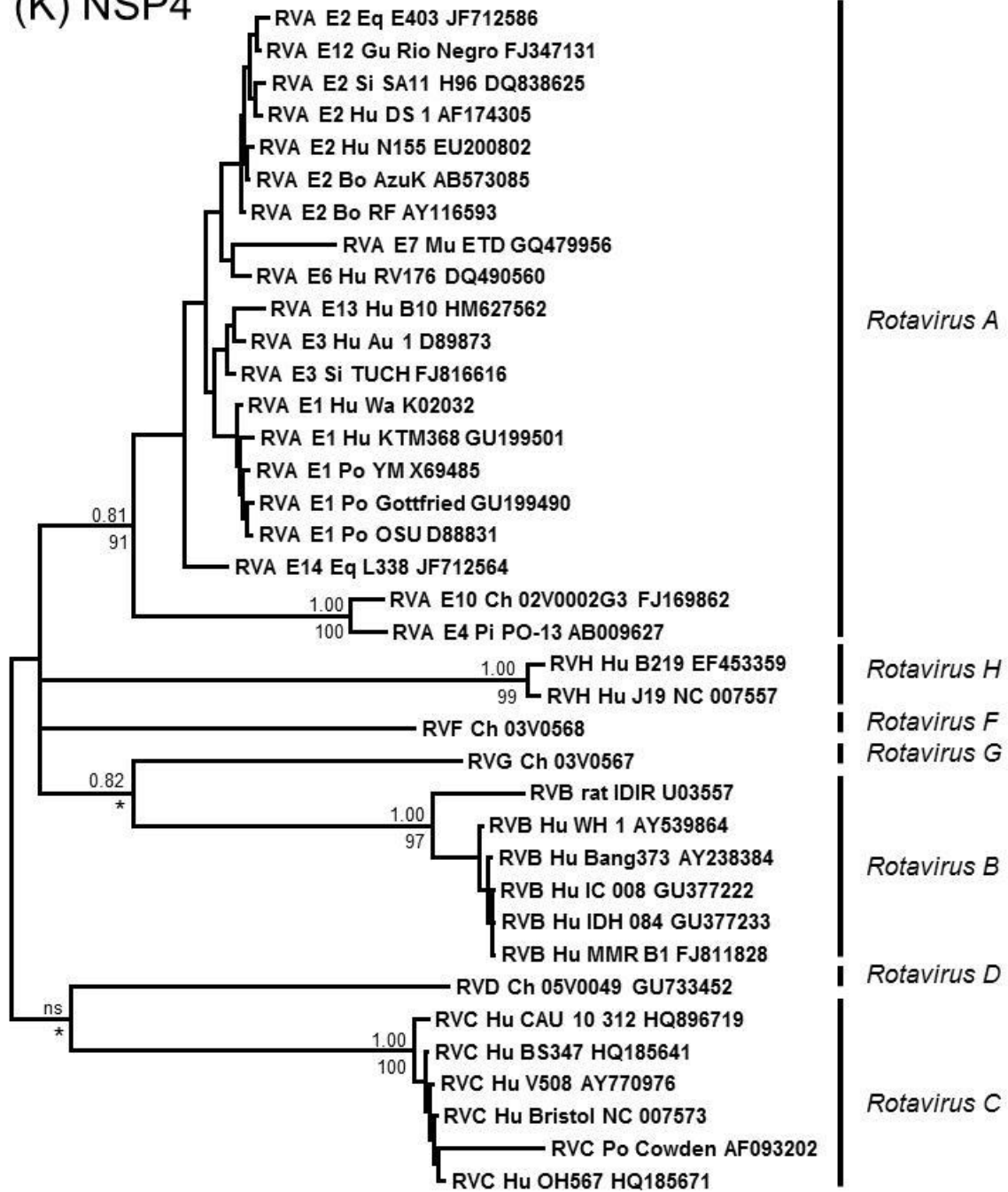
(H) NSP2



(I) NSP3

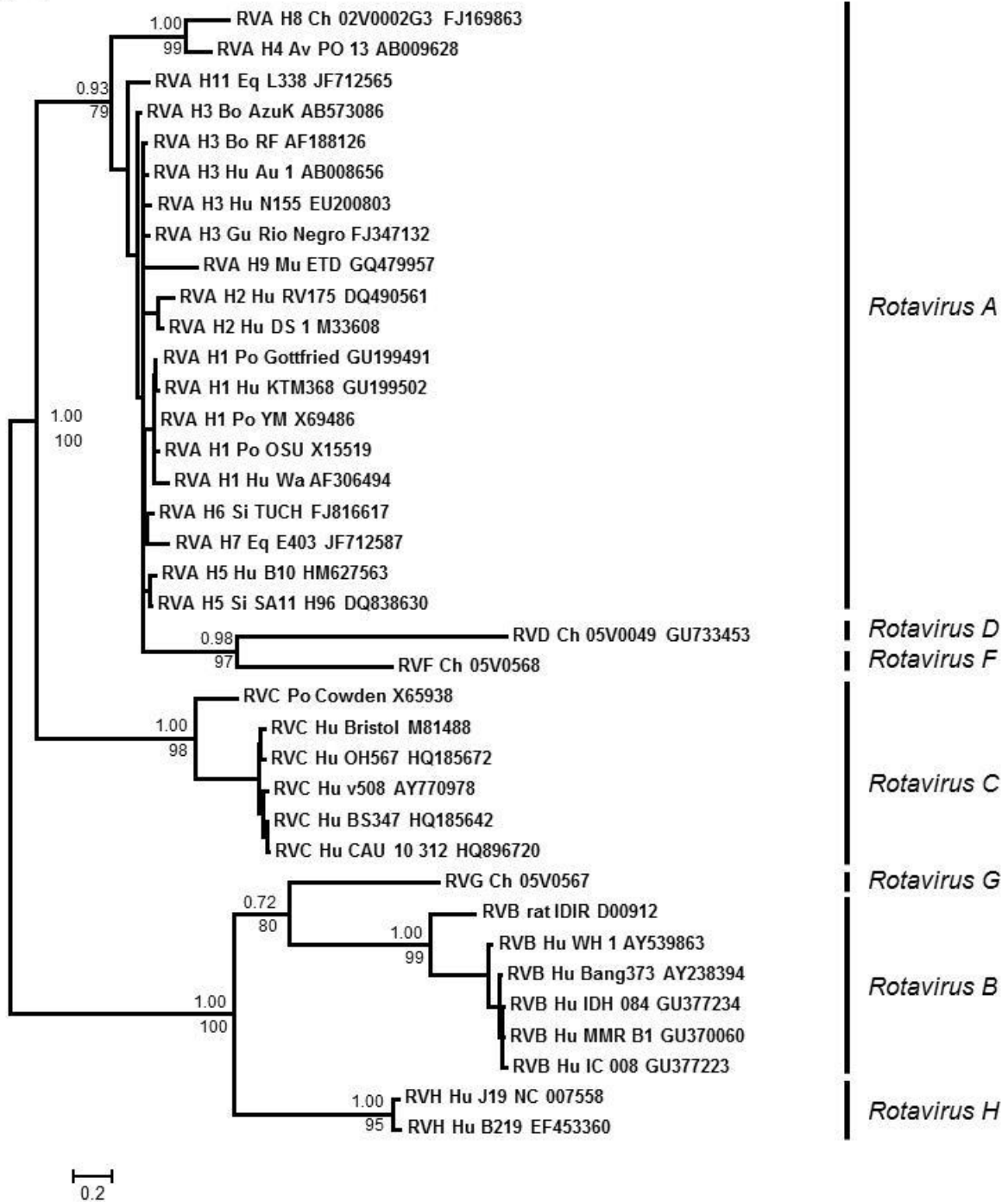


(K) NSP4



0.2

# (L) NSP5



## References

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