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:	***2014.011a-dV***	(to be completed by ICTV officers)

Short title: Create a new genus, *Reptarenavirus*, comprising three new species in the family *Arenaviridae*

(e.g. 6 new species in the genus *Zetavirus*)

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

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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)	ICTV *Arenaviridae* Study Group (Michael Buchmeier, Remi Charrel, Christopher S. Clegg, Sebastien Emonet, Jean-Paul Gonzalez, Igor S. Lukashevich, Clarence J. Peters, Sheli R. Radoshitzky, Victor Romanowski, Maria S. Salvato, Joseph L. DeRisi, Mark D. Stenglein, and Juan C. de la Torre)

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

EC Comments: The decision reached was Uc. This means that some revisions are requested but that if the proposal is modified satisfactorily, it can be re-considered by electronic vote of EC members in about 3 months' time, before proceeding to ratification.

The specific concerns were:

1. The proposal should provide a better phylogenetic tree with relevant outgroups and bootstrap values. Something along the lines of Figure 9 (page 741 of the 9th report) would support placing the new genus in the *Arenaviridae*

Response by the ASG: Phylogenetic trees are by nature unrooted, unless the investigator selects a sequence as a root before the analysis. Since the root will be used during the analysis to determine the ancestral states, it is a strong hypothesis that will clearly affect the final result. However, one can only guess which virus to use as a root for arenaviruses, since its sister-group is unknown. Therefore, using the wrong sequence may produce a false topology, and this error may be even more drastic when sequences are used that are much less conserved than the polymerase core protein we have used. Finally, if you consider the tree from the figure provided as an example and the tree found in Vieth *et al*., 2004 in *Virology* (PMID: 14972544), you will see that in one case, the root would be Dugbe virus (a nairovirus of the family *Bunyaviridae*), whereas in the other case, it would be better to use an orthomyxovirus.

Comments by the EC to the ASG Response: The following is a comment from one of the EC members which, I believe explains more precisely the original request.
"It is instructive to inspect Fig. 6 in the Virology reference provided by the authors (assuming that this analysis is valid). In this L-based tree, Arenaviridae forms a small monophyletic cluster within a much larger monophyletic cluster, whose other branches belong to bunyaviruses. Like bunyaviruses, the newly identified viruses in the submitted TP form also a lineage external to the currently known Arenaviridae. So our question to the authors was shall these new viruses be recognized as divergent arenaviruses or as a new branch of bunyaviruses? This could be answered with rooting by producing a tree including Bunyaviridae, Arenaviridae and the newly determined viruses. If these new viruses form a branch intermediate between Dugbe (Nairo) and Arenaviridae, then it is up to the authors to decide where to draw a demarcation border between two families effectively deciding in which family to place these new viruses. However, if these new viruses form a lineage basal to Nairo/Arenaviridae, then they must be recognized as bunyaviruses. So in conclusion, we are not questioning the distant relation of these new viruses to arenaviruses, but wondering could they be recognized as a separate subfamily (rather than a genus) in Arenaviridae or even Bunyaviridae"
The EC recognizes the problems associated with deeper phylogenies but, in order for the proposal to receive approval from the EC, I am sure that this question will have to be addressed. Of course, I can return the proposal to the EC as it now is but the most likely outcome is that some EC members will suggest it has to be discussed again at the next EC meeting.

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

Response by the ASG: We think there may be a misunderstanding that may have emerged during initial ICTV EC discussions of TP 2014.011. The assignment of the novel viruses in question into the family *Arenaviridae* was based on the fact that these viruses exhibit characteristics typical for already classified members in that family, all of which had been outlined in TP 2014.011, and, in more detail, in the referenced primary literature. Briefly:

- Like classic arenaviruses, the snake viruses have bi-segmented, negative strand RNA genomes (bunyaviruses have trisegmented genomes)
- Like classic arenaviruses, the snake viruses have genomes that encode four proteins. The S segment encodes NP and GPC proteins and the L segment encodes L and Z proteins (in bunyaviruses, the S segment encodes the N protein, the M segment encodes the GP protein, and the L segment encodes L – in contrast to arenaviruses, bunyaviruses encode nonstructural proteins NS_x; in contrast to arenaviruses, bunyaviruses do not encode a Z protein; and arenaviruses encode class I fusion glycoproteins, whereas bunyaviruses encode class II fusion glycoproteins)
- Like classic arenaviruses, the snake viruses employ an ambisense codon strategy for the same pairs of proteins, i.e. the S segment encodes the NP and GPC proteins in opposite directions and the L segment encodes the L and Z proteins in opposite directions (ambisense coding exists only among two of five bunyavirus genera, *Nairovirus* and *Tospovirus*, and in these viruses the proteins that are encoded in ambisense direction are the nonstructural proteins that have no equivalents in arenaviruses and ambisense coding is restricted to only one of the three genomic segments: S)
- Like classic arenaviruses, the snake arenaviruses contain unique non-coding intergenic hairpins, and conserved terminal sequences (in bunyaviruses, such hairpins are not present, and none of the bunyavirus genus-typical terminal genome segment sequences are shared with those of classical arenaviruses or the snake viruses).

Together, these characteristics clearly place the snake viruses among arenaviruses and strongly argue against a bunyavirus assignment. Moreover, there is no other virus group other than the classic arenaviruses that combines the features described above.

The ASG therefore focused the proposal on the question whether the snake viruses could be considered as all other known classic arenaviruses and should be placed within the same common genus, or whether a new genus with new species needed to be established within the family *Arenaviridae* to account for the distinct features exhibited by the snake arenaviruses. To address this issue, the ASG focused on the outcomes of L and NP phylogenetic analyses and PASC, and the results from these analyses were supplemented with biological data:

- The L and NP genes of the snake viruses are clearly, but distantly, related evolutionarily to those of the classic arenaviruses. Although the L proteins of most (-)ssRNA virus families are related, L genes of the snake viruses are more closely related to the classic arenavirus L genes than they are to L genes from other (-)ssRNA viruses. The NP gene does not cluster with functional equivalents in other virus families, including bunyaviruses. Moreover, the Z

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

protein has no functional equivalent in other virus families, including bunyaviruses.

- The sequence similarity between the snake viruses and classic arenaviruses is about the same as the inter-genus similarity for other RNA virus families. This is supported by the PASC profiles for arenaviruses and other RNA virus families. Virus sequences from different genera share ~25% identity by the local BLAST alignment measure and ~50% identity by the global alignment measure. Therefore, the sequence-based demarcation criteria we have used to establish the two arenavirus genera are consistent with criteria used for other RNA virus families. Examples of RNA virus families with similar degrees of inter-genera sequence similarity include *Astroviridae*, *Caliciviridae*, *Filoviridae*, *Rhabdoviridae*, and *Flaviviridae*.
- The organization and structure of the snake virus GPC protein is unique compared to classic arenavirus GPC proteins.
- The snake viruses infect snakes, whereas all known classic arenaviruses infect mammals.

We therefore concluded that the snake viruses represent a new genus in the family *Arenaviridae*. Because there are only a total of two genera under discussion (one for classic arenaviruses and one for snake viruses), the creation of subfamilies does not appear to make sense at this point in time.

All the points cited in this response were previously presented in TP 2014.011 and in the literature referenced in TP 2014.011. We therefore consider that this proposal can be accepted by the ICTV EC as it currently stands.

2. The proposal should include the relevant PASC diagram(s) and a more detailed description of the methodology. For example, what is the reliability of PASC analysis done at the protein level, using 30-35% pairwise identity, as a criterion to demarcate genera in the family

Response by the ASG: To address this issue we have incorporated additional information in section “species demarcation criteria in the new genus”, including links to the appropriate web sites that provide detailed descriptions of the PASC methodology and results, as well as an image of the relevant PASC diagram as a new Figure 2.

Comments by the EC to the ASG Response: An ever increasing number of proposals are using the PASC analysis to define taxon demarcation criteria. The ICTV has never tried to dictate any specific methodology in modelling phylogeny and, therefore, I am prepared to accept the modifications and recommend to the EC that the proposal is approved in this respect. However, I should tell you that some members of the EC are reluctant to accept the validity of the PASC approach (or, indeed, any taxonomy based upon degrees of divergence between whole or partial genome sequences) and wish to see additional biological characteristics taken into account. This debate is, however, for the future, I hope.

Response by the ASG: We are delighted that the Subcommittee Chair is prepared to accept the modifications and to recommend to the EC that it is approved.

We agree with the EC that clear guidelines need to be established in regard to

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

which methods ought to be used for classification (or more importantly, which ones are unacceptable) and are looking forward to having these discussions. The ASG worked under guidance of the ICVCN, which does not stipulate methods for classification. Because the ICTV Study Groups are composed of the experts for individual virus families it is expected that they would be responsible for developing the most suitable classification methods based on their expertise. Based on the ICVCN, the ASG assumed that the responsibility of the EC is to ensure that proposed classifications and nomenclatures are not at odds with the ICVCN Rules. We have developed the proposed arenavirus taxonomy based on several methodologies (PASC analyses, BLAST analyses, phylogenetic analyses of NP and L genes, genome structure comparisons, and biological characteristics – see also our recently submitted review article, which we have attached here for the EC's information). Therefore, we think that the proposed classification is the best currently possible, and we are not aware of which other methods could have been superior or would have produced significantly different results.

Date first submitted to ICTV:	6/2/2014
Date of this revision (if different to above):	23/10/2014

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	***2014.011aV***	(assigned by ICTV officers)

To create three (3) new species within:

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.

Genus:	***Reptarenavirus* (new)**
Subfamily:	
Family:	***Arenaviridae***
Order:	

Name of new species:	**Member virus(es):**	**GenBank sequence accession number(s)**
Alethinophid 1 reptarenavirus	Golden Gate virus	JQ717263.1, JQ717264.1
Alethinophid 2 reptarenavirus	California Academy of Sciences virus	JQ717262.1, JQ717261.1
Alethinophid 3 reptarenavirus	Boa AV NL B3 University of Helsinki virus	KC508669.1, KC508670.1 KF297880.1, KF297881.1

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

This proposal considers all published snake-infecting arenaviruses for which coding complete genome sequences are available (Stenglein *et al.*, 2012; Bodewes *et al.*, 2013; Hetzel *et al.*, 2013). There are four such viruses, tentatively named Golden Gate virus, California Academy of Sciences virus, Boa AV NL B3, and University of Helsinki virus.

The creation of novel species in the family *Arenaviridae* is currently based on the following species demarcation criteria established by the ICTV *Arenaviridae* Study Group:

- **an association with a specific host or group of hosts;**

Golden Gate virus, California Academy of Sciences virus, University of Helsinki virus, and Boa AV NL B3 have been isolated from boid snakes.

- **presence in a defined geographical area;**

  The geographical area is not yet defined; *B. constrictor* may not be the reservoir host.

- **etiological agent (or not) of disease in humans;**

  No evidence for human disease (people handling sick or deceased snakes have not contracted disease).

- **significant differences in antigenic cross-reactivity, including lack of cross-neutralization;**

  Hetzel *et al*., in 2013, reported viruses not to cross-react by western blot with anti-Junín virus antibodies, whereas weak cross reactivity was noted with anti-Machupo virus and anti-LCMV antibodies. The snake viruses do not react with antisera from patients with Bolivian hemorrhagic fever in ELISAs.

- **significant differences in nucleotide sequence.**

  Based on the species demarcation criteria described above, the four snake-associated viruses could be assigned to three distinct species to be included into the genus *Reptarenavirus* genus. By PASC analysis of L segment sequences, the viruses grouped within distinct species share less than 76% sequence similarity (Yiming Bao, unpublished). Boa AV NL B3 and University of Helsinki virus share >76% similarity by this measure (≈88%) and should therefore assigned to a single species.

The proposed naming scheme for these species takes the form of: ***Alethinophid X reptarenavirus*** (Alethinophidia: i.e., the Serpentes infraorder including species for most snakes). This naming scheme has the following advantages:

1. it follows ICVCN Rules for species nomenclature;
2. it is based on non-Latinized binomial names (van Regenmortel *et al*., 2010);
3. it is based on names that will not be identical to virus member names, therefore decreasing possible confusion of taxa and viruses.
4. it is systematic and extendable; new species names can be generated simply.
5. use of "alethinophid" does not suggest an overly specific host association and thereby it guarantees stability of species names over time (new snake arenaviruses that have yet to be published have been found in snakes not belonging to the family Boidae; Stenglein & deRisi, unpublished data).

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2014.011bV***	(assigned by ICTV officers)
To create a new genus within:		
Subfamily:		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 family is specified, enter "**unassigned**" in the family box
Family:	***Arenaviridae***	
Order:		

naming a new genus

Code	***2014.011cV***	(assigned by ICTV officers)
To name the new genus: *Reptarenavirus*		

Assigning the type species and other species to a new genus

Code	***2014.011dV***	(assigned by ICTV officers)
To designate the following as the type species of the new genus		
Alethinophid 1 reptarenavirus		Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered
The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**		
3		

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

The recent discovery of arenavirus-like viruses in snakes meant that the notion that arenaviruses can only infect mammals has to be revised (Stenglein *et al*., 2012).

The initial publication on these viruses included the complete genomic characterization of two distinct US viruses, a description of the isolation and propagation in culture of one of these viruses, and the establishment of an association between infection and a clinical diagnosis of snake inclusion body disease (Stenglein *et al*., 2012). Subsequent papers have described additional distinct viruses infecting snakes in Europe and examined additional aspects of the biology of these viruses (Bodewes *et al*., 2013, 2014; Hetzel *et al*., 2013, 2014).

Several characteristics of these snake viruses justify their inclusion in the family *Arenaviridae* (Stenglein *et al*., 2012), including:

1) the genomes of these viruses have the typical arenavirus genome organization: they consist of two genome segments (S and L), each containing two major open reading frames arranged in ambisense orientation;
2) the terminal sequences of the genome segments are similar (≈13/19 nucleotides) to those of previously described arenaviruses;
3) the viral nucleoprotein (NP) and RNA-dependent RNA polymerase (L) are clearly related evolutionarily to the cognate proteins of mammal-infecting arenaviruses (**Fig. 1**).

Several features distinguish these snake-infecting viruses from previously described arenaviruses (Stenglein *et al*., 2012) and justify their placement in a new genus to be included in the family *Arenaviridae*:

1) although the full extent of their host range remains to be determined, existing data support the conclusion that these viruses infect snakes and not mammals;
2) the viral NP and L proteins form a distinct evolutionary lineage in phylogenies, and only share ≈20-25% sequence similarity with Old and New World (mammalian) arenavirus homologs (**Fig. 1**);
3) neither the putative viral "Z" protein nor the GP2 domains of the viral glycoprotein possess detectable sequence similarity to Old and New World (mammalian) arenavirus homologs. GP2 is more closely related evolutionarily to GP2 domains of filoviruses and some avian retroviruses (Koellhoffer *et al*., 2014) (**Fig. 1)**;
4) pairwise sequence comparison (PASC) analysis indicates that these viruses should be placed in a new genus in the *Arenaviridae* family.

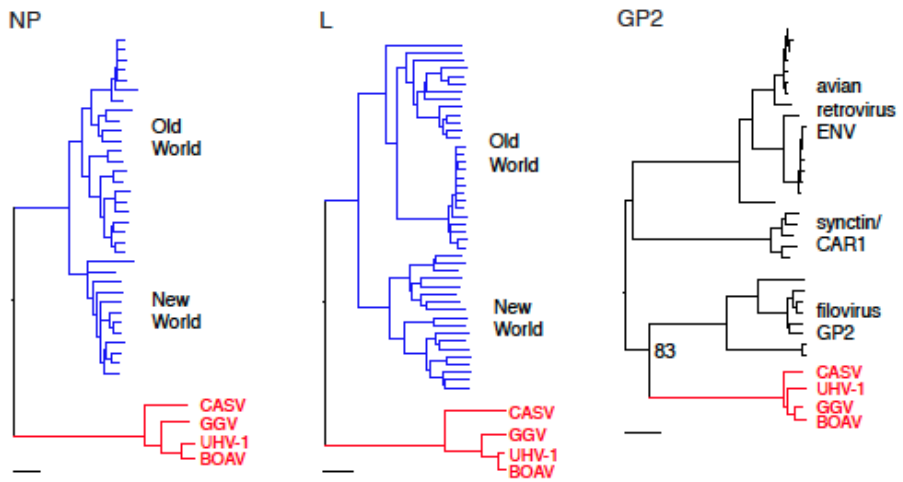

Figure 1: Maximum-likelihood phylogenies of predicted snake virus nucleoprotein (NP), polymerase (L), and glycoprotein GP2 domain protein and related sequences. Old World and New World designate major clades of previously described mammalian arenaviruses. Scale bars indicate 0.2 substitutions per site. The bootstrap percentages for select nodes are indicated. Figure adapted from (Stenglein *et al*., 2012); copyright retained by authors under Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported license.

Origin of the new genus name:

The proposed name for this new genus is ***Reptarenavirus***, a sigil of "reptile" and "arenavirus." This name has the following advantages:

1. it takes the form *Xxxvirus*, complying with ICVCN Rules for genus nomenclature;
2. it is simple, descriptive, and unique;
3. it is easy to pronounce (is euphonious);
4. *Reptarenavirus* preserves the word stems of the old virus genus (*Arenavirus*) but makes no reference (such as "boid") to any host taxon name. The proposed new *Mammarenavirus* genus to accommodate the arenavirus infecting mammals (replacing the current *Arenavirus* genus) is consistent with this etymology (see proposal XXX-2 submitted in parallel). It is considered unlikely that an arenavirus species would have to be reassigned from the genus *Mammarenavirus* to the genus *Reptarenavirus*;
5. the name *Reptarenavirus* does not suggest an overly specific host range. For example, the use of the prefix "Boid" would suggest that these viruses only infect snakes in the family Boidae (boas), but arenaviruses from snakes have by now been isolated from snakes of the family Pythonidae (pythons; unpublished data), and the full extent of the host range of reptarenaviruses therefore remains an open question;
6. the name *Reptarenavirus* does not suggest an unsubstantiated role in disease causality. Although there is strong evidence associating infection by these viruses with clinical diagnosis of inclusion body disease (Stenglein *et al*., 2012; Bodewes *et al*., 2013; Hetzel *et al*., 2013), formal demonstration of disease causality (i.e.. fulfillment of Koch's postulates) has not been reported, and the recent discovery of dozens of new snake arenaviruses, some from apparently healthy snakes, (Stenglein *et al*., unpublished) suggests that infection is not necessarily associated with clinical disease.

Reasons to justify the choice of type species:

There are several reasons to justify the selection of the species *Alethinophid 1 reptarenavirus* as the type species for the genus *Reptarenavirus*. First, at a sequence level the genome of Golden Gate virus, which is a proposed member of this species, possesses characteristics typical of viruses in the genus, including length and gene organization and orientation. Second, Golden Gate virus is the best-characterized snake arenavirus and the one for which the most laboratory reagents and systems exist. Sanger sequencing and rapid amplification of cDNA ends (RACE) were used to fully corroborate the sequencing-based assembly of the Golden Gate virus genome. Third, Golden Gate virus was one of the first two snake arenaviruses to be characterized, and was the first to be isolated in tissue culture. A polyclonal antibody targeting the GGV NP is available (Stenglein et al, 2012).

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Pairwise sequence comparison (PASC) analysis was performed on the L segment sequences of viruses in this genus to identify species and genus demarcation criteria (Yiming Bao, unpublished). This analysis strategy uses all-*vs*.-all pairwise alignments to identify logical cutoff thresholds (Bao *et al*., 2008). This tool was developed and has been used previously for this purpose (see for example Bao *et al*., 2012).

Based on this analysis, a species demarcation cutoff of <80% pairwise nucleotide similarity for S segment and <76% for L segment sequences is proposed. Example: two viruses with L segment

sequences sharing 70% pairwise identity by PASC analysis would be assigned to different species; and two viruses with L segments sharing 82% pairwise similarity by this measure would be assigned to a single species.

This analysis also defined a criterion to demarcate genera within the family *Arenaviridae*: clades of viruses with L-segment sequences that share less than 30-35% pairwise identity should be grouped into distinct genera.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Bao, Y., Kapustin, Y., and Tatusova, T. (2008). Virus Classification by Pairwise Sequence Comparison (PASC). In Encyclopedia of Virology (Third Edition), B.W.J. Mahy, and M.H.V.V. Regenmortel, eds. (Oxford: Academic Press), pp. 342–348.

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Bodewes, R., Kik, M.J.L., Raj, V.S., Schapendonk, C.M.E., Haagmans, B.L., Smits, S.L., and Osterhaus, A.D.M.E. (2013). Detection of novel divergent arenaviruses in boid snakes with inclusion body disease in The Netherlands. J. Gen. Virol. 94, 1206–1210.

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Koellhoffer, J.F., Dai, Z., Malashkevich, V.N., Stenglein, M.D., Liu, Y., Toro, R., Harrison, J., Chandran, K., DeRisi, J.L., Almo, S.C., and Lai, J.R. (2014). Structural characterization of the glycoprotein GP2 core domain from the CAS virus, a novel arenavirus-like species. J. Mol. Biol. 426, 1452-1468.

Hetzel, U., Sironen, T., Laurinmäki, P., Liljeroos, L., Patjas, A., Henttonen, H., Vaheri, A., Artelt, A., Kipar, A., Butcher, S.J., et al. (2013). Isolation, identification, and characterization of novel arenaviruses, the etiological agents of boid inclusion body disease. J. Virol. 87, 10918–10935.

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Stenglein, M.D., Sanders, C., Kistler, A.L., Ruby, J.G., Franco, J.Y., Reavill, D.R., Dunker, F., and Derisi, J.L. (2012). Identification, characterization, and in vitro culture of highly divergent arenaviruses from boa constrictors and annulated tree boas: candidate etiological agents for snake inclusion body disease. mBio 3, e00180–00112.

Van Regenmortel, M.H., Burke, D.S., Calisher, C.H., Dietzgen, R.G., Fauquet, C.M., Ghabrial, S.A., Jahrling, P.B., Johnson, K.M., Holbrook, M.R., Horzinek, M.C., Keil, G.M., Kuhn, J.H., Mahy, B.W., Martelli, G.P., Pringle, C., Rybicki, E.P., Skern, T., Tesh, R.B., Wahl-Jensen, V., Walker, P.J., and Weaver, S.C. (2010). A proposal to change existing virus species names to non-Latinized binomials. Arch. Virol. 2010 155, 1909-1919.

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.