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:	***2013.003a,bF***	(to be completed by ICTV officers)

Short title: Reconsideration of existing species in genus *Leishmaniavirus*, family *Totiviridae*
(e.g. 6 new species in the genus *Zetavirus*)

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

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

Max L. Nibert (mnibert@hms.harvard.edu); Said A. Ghabrial (saghab00@email.uky.edu); Jean L. Patterson (jpatters@TxBiomed.org); Nicolas Fasel (Nicolas.Fasel@unil.ch); Stephen M. Beverley (beverley@wustl.edu)

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)	*Totiviridae* Study Group

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

Totiviridae Study Group (SG): Stephen M. Beverley, Jeremy A. Bruenn, Øystein Evensen, Said A. Ghabrial, Tetsuya Mizutani, Max L. Nibert, Jean L. Patterson, Marilyn J. Roossinck, Drake C. Stenger, Ioannis Tzanetakis, Ching C. Wang, Reed B. Wickner, Iñigo Zabalgogeazcoa.

All SG members were provided an original version of this proposal, and several suggestions were incorporated into this current revision now being forwarded to the ICTV-EC.

ICTV-EC comments: Consider renaming species without use of 'RNA' in the names.

Response of proposers: Given the >20 years of precedent in this field for the name 'Leishmania RNA virus', and no strong argument besides simplification for dropping 'RNA' from this name, we request to maintain the new species names *Leishmania RNA virus 1* and *Leishmania RNA virus 2* as originally proposed.

Date first submitted to ICTV: June 2013
Date of this revision (if different to above):

MODULE 7: **REMOVE and MOVE**

Use this module whenever an existing taxon needs to be removed:
- *Either* to abolish a taxon entirely (when only part (a) needs to be completed)
- *Or* to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	***2013.003aF***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

Leishmania RNA virus 1-2
Leishmania RNA virus 1-3
Leishmania RNA virus 1-4
Leishmania RNA virus 1-5
Leishmania RNA virus 1-6
Leishmania RNA virus 1-7
Leishmania RNA virus 1-8
Leishmania RNA virus 1-9
Leishmania RNA virus 1-10
Leishmania RNA virus 1-11
Leishmania RNA virus 1-12

The present taxonomic position of these taxon/taxa:

Genus:	***Leishmaniavirus***
Subfamily:	
Family:	***Totiviridae***
Order:	

Fill in all that apply.

If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right	**YES**

Reasons to justify the removal:
Explain why the taxon (or taxa) should be removed

Leishmaniavirus is one of 5 exisiting genera in the family *Totiviridae*, approved members of which to date are encapsidated dsRNA viruses of fungal and protozoan hosts. An introductory phylogram is shown in **Figure 1** to illustrate these points. Although genus *Leishmaniavirus* currently comprises 13 approved species, full-length genome sequences have been reported for only the 3 species represented by strains in **Figure 1**: *Leishmania RNA virus 1-1*, *Leishmania RNA virus 1-4*, and *Leishmania RNA virus 2-1*.

Listed above as the main topic of this taxonomic proposal are the 10 other approved species in genus *Leishmaniavirus*, as well as *Leishmania RNA virus 1-4*. As supported by evidence below, the reference strains of these 11 listed species appear all to belong to the same species as the reference strain of *Leishmania RNA virus 1-1*, not to 12 different species. We therefore propose to eliminate the 11 species listed above, leaving *Leishmania RNA virus 1-1* and *Leishmania RNA virus 2-1* as the only 2 approved species in this genus.

Supporting evidence
Full-length genome sequences have been reported for the reference strains of both

Leishmania RNA virus 1-1 and *Leishmania RNA virus 1-4* (Stuart et al., 1992; Scheffter et al., 1994), and pairwise comparisons between these two strains show nt-and aa-identity scores of 77.5%, 90.7%, 82.0%, and 86.0% for their genome, CP, RdRp, and CP+RdRp sequences, respectively. These pairwise scores are much too far above the suggested threshold of 50% in family *Totiviridae* to warrant separation of these two viruses into different species.

For the 10 other approved species listed above, partial genome sequences (~250 nt) from the plus-strand 5´ untranslated region have been reported for reference strains of 6 of them: *Leishmania RNA virus 1-2*, *Leishmania RNA virus 1-7*, *Leishmania RNA virus 1-8*, *Leishmania RNA virus 1-9*, *Leishmania RNA virus 1-10*, and *Leishmania RNA virus 1-11* (Zamora et al., 2000), and pairwise comparisons of this ~250-nt region from these strains, as well as the reference strains of *Leishmania RNA virus 1-1* and *Leishmania RNA virus 1-4*, yield high identity scores of 85.3–96.8% (**Table 1**).

In phylogenetic sequence comparisons, the co-clustering of all these strains further indicates their close relatedness, as well as their more distant relationship to the reference strain of *Leishmania RNA virus 2-1* (**Figure 2**). Although the trees seem to suggest two subclades among the different "LRV1" viruses, the extent of divergence is not great enough to warrant division into two separate species reflecting these subclades. For example, the *Leishmania RNA virus 1-1* and *Leishmania RNA virus 1-2* reference strains, from the different apparent "LRV1" subclades suggested in **Figure 1**, have almost the same pairwise identity score for their plus-strand 5´ untranslated region (90.5%) as do the *Leishmania RNA virus 1-1* and *Leishmania RNA virus 1-4* reference strains, from the same apparent "LRV1" subclade (90.4%) (**Figure 2; Table 1**).

Based on these consistent findings, we conclude that the reference strains of existing species *Leishmania RNA virus 1-2*, *Leishmania RNA virus 1-4*, *Leishmania RNA virus 1-7*, *Leishmania RNA virus 1-8*, *Leishmania RNA virus 1-9*, *Leishmania RNA virus 1-10*, and *Leishmania RNA virus 1-11* all belong to the the same species as the reference strain of *Leishmania RNA virus 1-1*, and should therefore be removed from the list of approved species in genus *Leishmaniavirus*. We propose that the 3 approved species in the list above for which no strain sequences are available—*Leishmania RNA virus 1-3*, *Leishmania RNA virus 1-5*, and *Leishmania RNA virus 1-12*—should also be removed, because the validity of their species status cannot be evaluated in the absence of sequence data and also because these viruses were isolated at similar geographic locations and times as the other "LRV1" viruses and are thus likely to belong as well to the same species as do the other "LRV1" viruses.

In contrast, the reference strain of approved species *Leishmania RNA virus 2-1*, for which a full-length genome sequence is also available (Scheffter et al., 1995), appears in the phylogenetic tree on a well-separated branch from the "LRV1" viruses discussed above (**Figure 2**), consistent with its continued recognition as a distinct species. Moreover, pairwise comparisons show respective nt- or aa-identity scores of only 49.4%, 36.0%, 40.1%, and 38.7% for the genome, CP, RdRp, and CP+RdRp sequences of the *Leishmania RNA virus 2-1* vs. *Leishmania RNA virus 1-1* reference strains, consistent with the continued recognition of both these existing species. Also see **Table 1** for results indicating the similarly greater divergence of LRV2-1 from "LRV1" strains in the 250-nt 5´ untranslated region discussed above. **Table 2** summarizes a few genomic and coding properties in which the reference strain of *Leishmania RNA virus 2-1* is distinctive, especially the lack of an overlap between ORF1 and ORF2. Lastly worth noting is that the reference strain of

Leishmania RNA virus 2-1 derives from Old World host species *Leishmania major*, whereas the reference strains of *Leishmania RNA virus 1-1* and the others discussed above derive from New World host species *Leishmania braziliensis* or *Leishmania guyanensis*.

In conclusion, we propose in this module to eliminate the 11 existing species listed above, leaving only 2 existing species in genus *Leishmaniavirus*: *Leishmania RNA virus 1-1* and *Leishmania RNA virus 2-1*. Module 8 below concerns name changes to these two remaining species.

MODULE 8: **NON-STANDARD**

Template for any proposal not covered by modules 2-7. This includes proposals to change the name of existing taxa (but note that stability of nomenclature is encouraged wherever possible).

non-standard proposal

Code	***2013.003bF***	(assigned by ICTV officers)

Title of proposal: In genus *Leishmaniavirus*, change the name of species *Leishmania RNA virus 1-1* to *Leishmania RNA virus 1* and of species *Leishmania RNA virus 2-1* to *Leishmania RNA virus 2*

Text of proposal:

After removing 11 of the 13 approved species in genus *Leishmaniavirus*, as proposed in Module 7 above, the 2 species that remain are *Leishmania RNA virus 1-1* and *Leishmania RNA virus 2-1*. These 2 species need to be renamed, however, because the "*-1*" in each of their names is a strain designation. We therefore propose to change these respective species names to *Leishmania RNA virus 1* and *Leishmania RNA virus 2*.

In addition, since *Leishmania RNA virus 1-1* is the current type species of genus *Leishmaniavirus*, the type species will become *Leishmania RNA virus 1* after the name change.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Scheffter SM, Ro YT, Chung IK, Patterson JL. 1995. The complete sequence of Leishmania RNA virus LRV2-1, a virus of an Old World parasite strain. *Virology* **212,** 84–90.

Scheffter S, Widmer G, Patterson JL. 1994. Complete sequence of Leishmania RNA virus 1-4 and identification of conserved sequences. *Virology* **199,** 479–83.

Stuart KD, Weeks R, Guilbride L, Myler PJ. 1992. Molecular organization of Leishmania RNA virus 1. *Proc Natl Acad Sci USA* **89,** 8596–8600.

Widmer G, Dooley S. 1995. Phylogenetic analysis of Leishmania RNA virus and *Leishmania* suggests ancient virus-parasite association. *Nucleic Acids Res* **23,** 2300–2304.

Zamora M, Guilbride L, Sacks L, Stuart K. 2000. Phylogenetic analysis of the 5′ subterminal region of isolates of Leishmania RNA virus-1. *Ann Trop Med Parasitol* **94,** 123–133.

Zangger H, Ronet C, Desponds C, Kuhlmann FM, Robinson J, Hartley MA, Prevel F, Castiglioni P, Pratlong F, Bastien P, Müller N, Parmentier L, Saravia NG, Beverley SM, Fasel N. 2013. Detection of Leishmania RNA virus in *Leishmania* parasites. *PLoS Negl Trop Dis* **7,** e2006.

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.

Figure 1. Maximum-likelihood phylogenetic analysis of the CP+RdRp sequences of *Totiviridae* family members, highlighting the position of genus *Leishmaniavirus* (blue shading, boxed). Alignments were conducted using Clustal Omega version 1.1.0 as implemented at http://www.ebi.ac.uk/Tools/msa/clustalo/ with default settings. Trees were generated with PhyML 3.0 as implemented at http://www.hiv.lanl.gov/content/sequence/PHYML/interface.html using the LG substitution model, empirical equilibrium frequencies, program-estimated invariant-proportion value (0.007) and gamma-shape value (2.008), and 4 rate categories. The starting tree was obtained by BioNJ and optimized by both branch length and tree topology. Tree improvement was performed according to the best of nearest neighbor interchange and subtree pruning and regrafting. Branch support values (%) were estimated by the approximate likelihood ratio test (aLRT) with SH-like criteria; branches with support values ≥ 90% are unlabeled, and those with support values < 50% are collapsed to form polytomies. Recently proposed species of totiviruses are shown in red. Two probable species of totiviruses that have not been assigned to genera are represented by strains EbRV1 and IMNV. Two partitivirus sequences (AhV and WCCV1) were included as an outgroup, on which the tree is rooted. Other color and shading are explained in the figure. The putative host of each virus is shown at right: F, fungus; Pr, protozoan; A, arthropod; and P, plant.

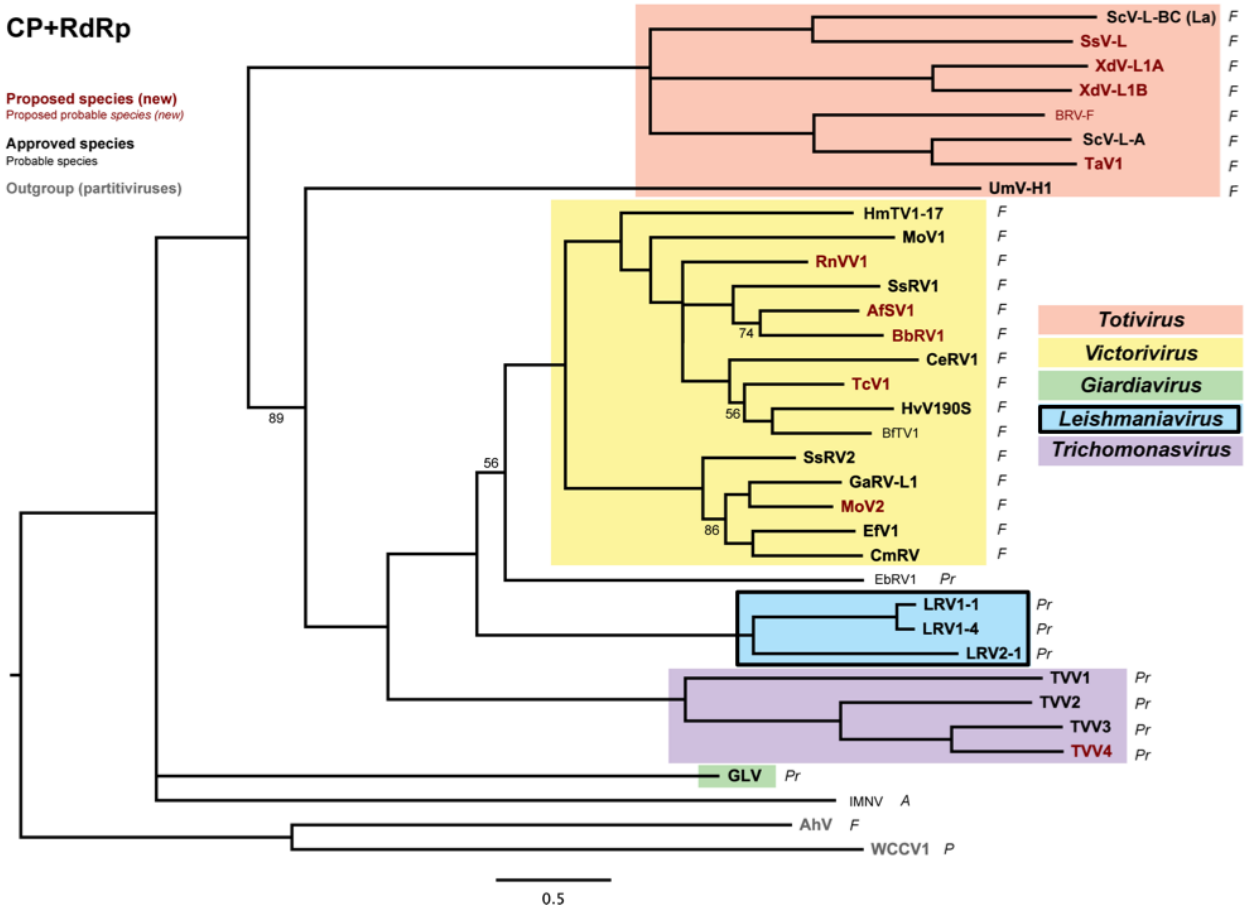

Figure 2. Maximum-likelihood phylogenetic analysis of the complete or partial genome sequences of *Leishmaniavirus* genus members. Alignments were conducted using Clustal Omega version 1.1.0 as implemented at http://www.ebi.ac.uk/Tools/msa/clustalo/ with default settings. Sequences for the *Leishmania RNA virus 1-1*, *Leishmania RNA virus 1-4*, and *Leishmania RNA virus 2-1* strains are complete (GenBank accession nos. M92355, U01899, and U32108) (Stuart et al., 1992; Scheffter et al., 1995, 1995); those for the *Leishmania RNA virus 1-2*, *Leishmania RNA virus 1-7*, *Leishmania RNA virus 1-8*, *Leishmania RNA virus 1-9*, *Leishmania RNA virus 1-10*, and *Leishmania RNA virus 1-11* strains are partial (GenBank accession nos. AF230881, AF230882, AF230883, AF230884, AF230885, and AF230886) (Zamora et al., 2000). Reported sequences for additional strains Leishmania RNA virus 1-13, Leishmania RNA virus 1-Lg1398, and Leishmania RNA virus 1-LgM5313 (GenBank accession nos. U23810, JX313126, and JX313127) (Widmer & Dooley, 1995; Zangger et al., 2013) are also included. Trees were generated with PhyML 3.0 as implemented at http://www.hiv.lanl.gov/content/sequence/PHYML/interface.html using the GTR substitution model, empirical equilibrium frequencies, program-estimated invariant-proportion value (0.000) and gamma-shape value (1.261), and 4 rate categories. The starting tree was obtained by BioNJ and optimized by both branch length and tree topology. Tree improvement was performed according to the best of nearest neighbor interchange and subtree pruning and regrafting. Branch support values (%) were estimated by the approximate likelihood ratio test (aLRT) with SH-like criteria. A trichomonasvirus (TVV1-UH9) genome sequence (GenBank accession no. HQ607516) was included as an outgroup.

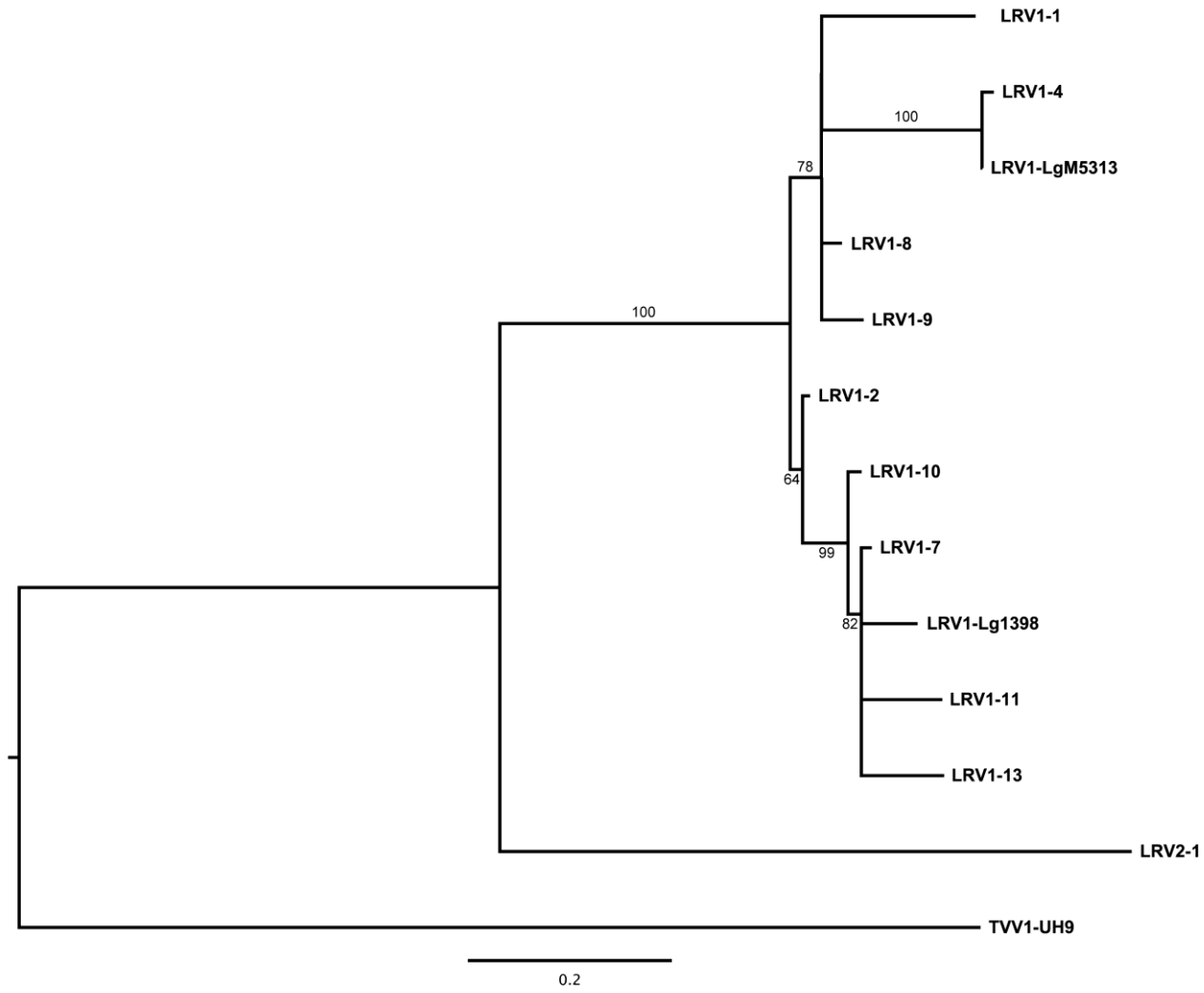

Table 1. Pairwise identity scores (%) for *Leishmaniavirus* genus members*

Virus	LRV1-1	LRV1-2	LRV1-4	LRV1-7	LRV1-8	LRV1-9	LRV1-10	LRV1-11	LRV2-1
LRV1-1	100	90.5	90.4	87.6	91.7	91.3	89.6	**85.3†**	45.0
LRV1-2		100	89.7	92.9	94.9	92.5	94.1	89.0	42.9
LRV1-4			100	88.8	91.7	91.7	89.6	86.5	42.5
LRV1-7				100	88.9	89.7	**96.8**	92.4	44.6
LRV1-8					100	96.8	90.1	89.0	44.0
LRV1-9						100	90.9	88.2	43.6
LRV1-10							100	91.2	43.0
LRV1-11								100	42.9
LRV2-1									100

* Sequences were first aligned using Clustal Omega. The longer sequences available for LRV1-1, LRV1-4, and LRV2-1 were then parsed to the 5′ untranslated region for which sequences are available for the other viruses. Identity scores for this common region were then determined using EMBOSS 6.3.1 needleall for pairwise global alignments (Blosum62 matrix, gap opening penalty 10, gap extension penalty 0.5). See Figure 1 for GenBank accession nos.
† Highest and lowest scores among the "LRV1" viruses are highlighted with bolding and underlining.
‡ Values for LRV2-1 are highlighted with shading.

Table 2. Properties of Leishmania RNA virus strains for which full-length sequences have been reported

Name	Genome (bp)	5′ UTR (nt)	3′ UTR (nt)	ORF1CP* (aa)	ORF2/RdRp† (aa)	ORF1-ORF2 overlap?
Leishmania RNA virus 1-1	5284	447	48	741	878	yes (fs+1)‡
Leishmania RNA virus 1-4	5283	449	43	742	878	yes (fs+1)‡
Leishmania RNA virus 2-1	5241	340	50	714	901	no§

* First Met codon to downstream stop codon
† Upstream stop codon to downstream stop codon
‡ Translation of CP/RdRp fusion by putative +1 ribosomal frameshifting
§ Mechanism of RdRp translation unknown