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:	*2014.013aV*	(to be completed by ICTV officers)

Short title: Two (2) new species in the genus *Arenavirus* (proposed separately to be renamed *Mammarenavirus*), family *Arenaviridae*
(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:

Bao, Yiming, bao@ncbi.nlm.nih.gov
Buchmeier, Michael J., m.buchmeier@uci.edu
Charrel, Remi, remi.charrel@univ-amu.fr
Clegg, Christopher S., cleggjcs@yahoo.fr
de la Torre, Juan Carlos, juanct@scripps.edu
Emonet, Sebastien, sebastien.emonet@irba.fr
Gonzalez, Jean-Paul, jean-paul.gonzalez@ird.fr
Kuhn, Jens H., kuhnjens@mail.nih.gov
Lukashevich, Igor S., isluka01@louisville.edu
Peters, Clarence J., cjpeters@utmb.edu
Radoshitzky, Sheli R., sheli.radoshitzky@ctr.army.mil
Romanowski, Victor, victor@biol.unlp.edu.ar
Salvato, Maria S., msalvato@ihv.umaryland.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)	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 Ac. This means that as with Uc some revisions are requested but, in this case, if the proposal is modified satisfactorily, as judged by me, it can proceed to ratification without the EC needing to see it again.

The specific concerns were:

1. Explain rooting of the phylogenetic tree. Would a reptilian arenavirus be appropriate?

Response by the ASG: This issue has been addressed in the new Figure 1 of the revised proposal, in which 1A presents the overall topology of the tree showing only the major clades including the reptilian clade and 1B presents the detailed topology for all the Old World arenavirus sequences used for the analysis.

Comments by the EC to the ASG Response: This has addressed the concerns regarding the rooting of the original tree

2. Add relevant PASC diagrams to the proposal.

Response by the ASG: This issue has been addressed in the new Figure 2 of the revised proposal.

Comments on the response by the EC: This has addressed the concerns.

Overall, therefore, I am moving TP 2014.013 from an Ac to an A (conditionally approved to approved) and it can now go forward for ratification. I am recommending that TP 2014.012 is changed from a Uc to an A (Under consideration and changes recommended to approved) but this will have to be confirmed by a vote of the EC (and I suspect there will be opposition). And finally, I am asking you to reconsider the more detailed EC comments regarding TP 2014.0011 (thus staying at Uc for the moment).

Response by the ASG: We are delighted and thank the Subcommittee Chair for accepting TP 2014.013.

Date first submitted to ICTV:	7/02/2014
Date of this revision (if different to above):	09/10/2014

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	*2014.013aV*	(assigned by ICTV officers)

To create 2 new species within:

Genus:	*Mammarenavirus* (new name for the former genus *Arenavirus*)	Fill in all that apply.
Subfamily:		• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Family:	*Arenaviridae*	• If no genus is specified, enter "**unassigned**" in the genus box.
Order:		

Name of new species:	Representative isolate:	GenBank sequence accession number(s)
Lunk mammarenavirus	Lunk virus, isolate NKS-1	S segment: AB693150 L segment: AB693151
Merino Walk mammarenavirus	Merino Walk virus, isolate Merino Walk	S segment: GU078660 L segment: GU078661

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 two published, novel Old World arenaviruses for which coding complete genome sequences for both S and L segments are available and which have been isolated.

The first virus was discovered in Zambia in southern African pygmy mice (*Mus* (*Nannomys*) *minutoides*), and named Lunk virus (Ishii *et al*., 2012).

The second virus was discovered in South Africa in bush Ka(r)roo rats (*Myotomys unisulcatus*) and named Merino Walk virus (Palacios *et al*., 2010).

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;**

   Lunk virus infects African pygmy mice, from which no other arenavirus has yet been isolated.

   Merino Walk virus infects bush Ka(r)roo rats, from which no other arenavirus has yet been isolated.

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

  > Lunk virus was discovered in Zambia, home of only only two other known arenaviruses (Luna virus and Lujo virus).

  > Merino Walk virus was discovered in South Africa, home of only one other known arenavirus (Luna virus)

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

  > There is no evidence for human disease caused by either virus.

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

  > Serological studies have not been performed with Lunk virus.

  > Palacios *et al*. (2012) detected a relationship of Merino Walk virus to the Old World arenaviruses Ippy virus, Mobala virus, and Mopeia virus, but not to lymphocytic choriomeningitis virus by CF testing. The closest relative of Merino Walk virus in this experiment was Mopeia virus.

- **significant differences in nucleotide sequence.**

  > Both Lunk and Merino Walk viruses share the arenavirus-typical ambisense and bisegmented genome organization.

  > Phylogenetic analyses based on partial NP and L genes place Lunk virus into the lymphocytic choriomeningitis virus clade as a distinct member. Pairwise sequence comparison (PASC) analysis, which uses all-*vs.*-all pairwise alignments to identify logical cutoff thresholds (Bao *et al*., 2008), was performed on both complete S and L segment sequences of Lunk virus and all other available coding complete arenavirus genomes. This analysis consistently placed Lunk virus into a new species when cut-offs were chosen that bring S and L segment results into alignment and uphold the current taxonomy of other family members (chosen species cut-off: 80% for the S segment and 76% for the L segment; Yiming Bao, unpublished; for results see: http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage=main&action=gilist&id=448 (S segment PASC) and http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage=main&action=gilist&id=446 (L segment PASC, also see Fig. 2). In this analysis, the closest relative of Lunk virus is lymphocytic choriomeningitis virus (in the S segment analysis 71% with WHI strain FJ607033; in the L segment analysis 64% with aggressive strain EU480451).

  > Phylogenetic analyses based on the NP, GP, and L genes place Merino Walk into the Ippy/Mobala/Mopeia virus clade as a distinct member. In the NP gene analysis, Merino Walk virus differs from the NP gene of the most closely related virus (Mopeia) by 34.5% (31.4% when the NP amino acid sequences are compared). PASC analysis was performed on both complete S and L segment sequences of Merino Walk virus and all other available coding complete arenavirus genomes. This analysis consistently placed Merino Walk virus into a new species when cut-offs were chosen that bring S and L segment results into alignment and uphold the current taxonomy of other family members (see links above for results). In this analysis, the closest relative of Merino Walk virus is Lassa virus (in the S segment analysis 64% with strain AV AF246121; in the L segment analysis 56% with strain Komina-R16 KF478760).

The naming scheme for the two proposed species takes the form of: ***Xxx mammarenavirus*** (*Mammarenavirus*: the genus included in the family *Arenaviridae* that harbors mammalian arenaviruses; see also corresponding Proposal that has been submitted).

Accordingly, Lunk virus would become a member of the mammarenaviral species *Lunk mammarenavirus*; and Merino Walk virus would become a member of the mammarenaviral species *Merino Walk mammarenavirus*.

MODULE 9: **<u>APPENDIX</u>**: 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.

Ishii, A., Thomas, Y., Moonga, L., Nakamura, I., Ohnuma, A., Hang'ombe, B.M., Takada, A., Mweene, A.S., and Sawa, H. (2012). Molecular surveillance and phylogenetic analysis of Old World arenaviruses in Zambia. J. Gen. Virol. 93, 2247-2251.
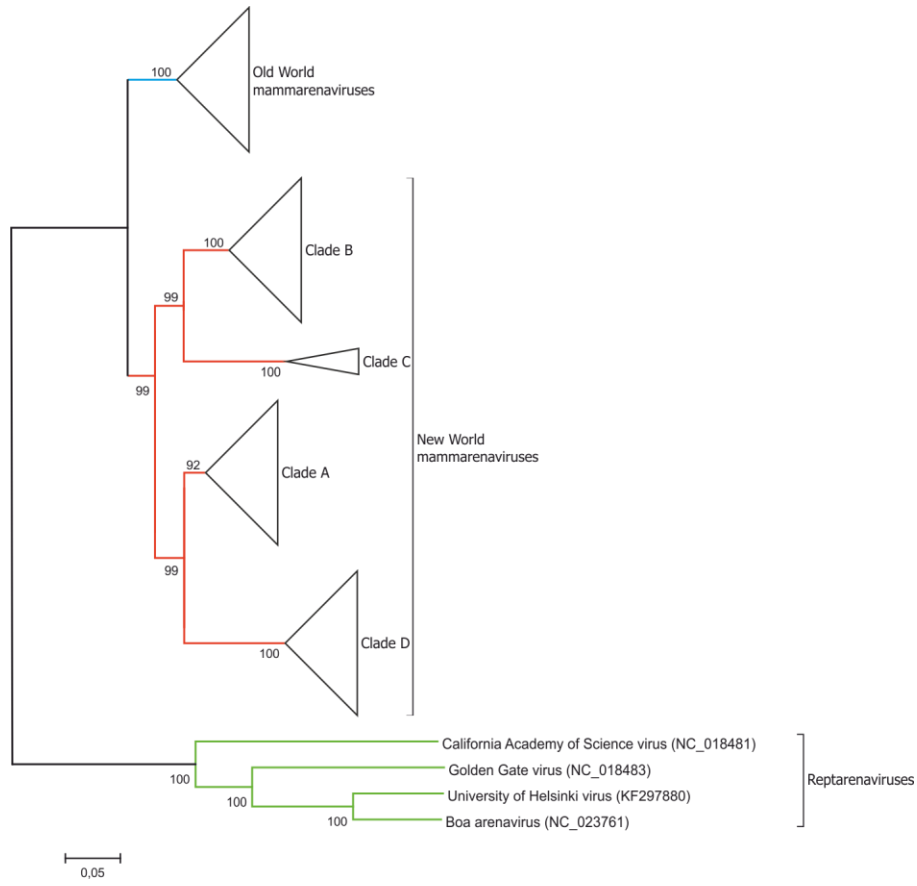
Palacios, G., Savji, N., Hui, J., Travassos da Rosa, A., Popov, V., Briese, T., Tesh, R., and Lipkin, W.I. (2010). Genomic and phylogenetic characterization of Merino Walk virus, a novel arenavirus isolated in South Africa. J. Gen. Virol. 91, 1315-1324.
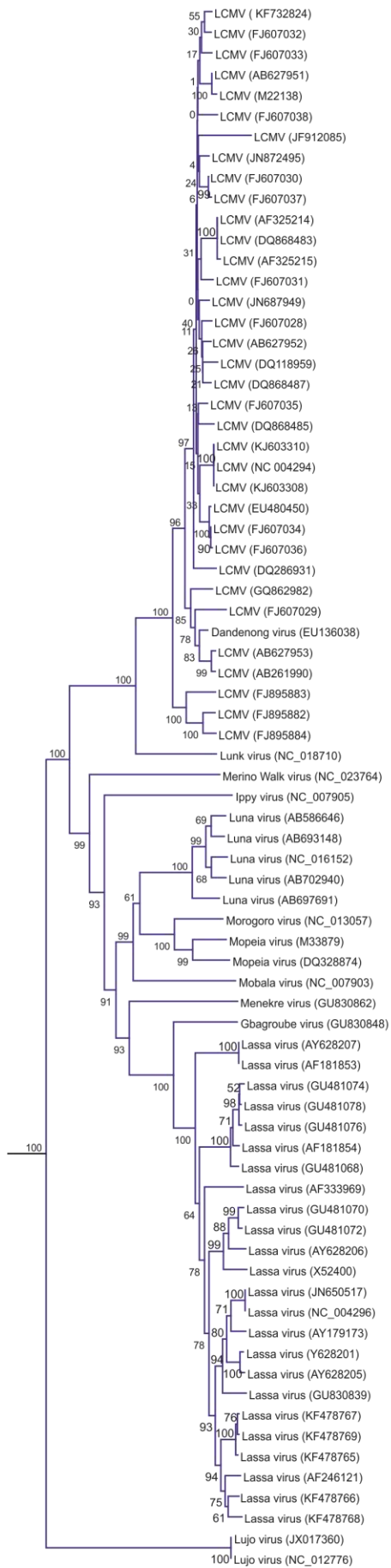
Annex:
Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with 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. Phylogeny of Old World mammarenaviruses based on the analysis of complete amino acid sequences of their encoded nucleoproteins. Amino acid sequences were aligned with the ClustalW implementation in the MEGA version 6 software. The phylogenetic tree was obtained using the Neighbor Joining analysis implemented into MEGA with the pairwise distance algorithm. Support for nodes was tested by bootstrapping using 1,000 pseudoreplications. A. Overall topology showing only the major clades. B. Detailed topology showing all the Old World sequences used.

A.

B.

Phylogenetic tree of Old World mammarenaviruses including LCMV, Dandenong virus, Lunk virus, Merino Walk virus, Ippy virus, Luna virus, Morogoro virus, Mopeia virus, Mobala virus, Menekre virus, Gbagroube virus, Lassa virus, and Lujo virus isolates with bootstrap support values.

Figure 2. Distribution of pairwise identities among 87 complete arenavirus L segment sequences. Regions A, B, and C represent virus pairs belonging to the same species, different species but the same genus, and different genera, respectively.