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:	***2012.009bV***	(to be completed by ICTV officers)

Short title: create 1 new species in the genus *Lyssavirus*
(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:

Conrad Freuling (Conrad.Freuling@fli.bund.de), Thomas Muller

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)	Rhabdovirus group

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

The submitted version was seen by the SG, and is supported with modifications.

Date first submitted to ICTV:	28/06/2012
Date of this revision (if different to above):	15/05/2013

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	***2012.009bV***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Lyssavirus***
Subfamily:	
Family:	***Rhabdoviridae***
Order:	***Mononegavirales***

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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Bokeloh bat lyssavirus	JF311903

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

Bokeloh bat lyssavirus (BBLV) was first isolated in mouse neuroblastoma cells (MNA) from the brain of a Natterer's bat (*Myotis nattereri*) that had died after a clinical disease suggestive of rabies in Germany during 2010. The presence of lyssavirus antigens was confirmed using direct immunofluorescence test (DFA) and immunohistochemistry with rabies biologics (Freuling et al., 2011). BBLV was pathogenic for mice via intracranial and intramuscular inoculation routes, causing fatal encephalitis. Kaplan-Meyer survival plots and clinical scores were significantly different for BBLV in comparison with EBLV-1 and EBLV-2. During infection, BBLV formed typical to lyssaviruses intracytoplasmic inclusions, detected by staining with FITC-conjugated anti-nucleocapsid monoclonal antibodies.

Besides the initial case, two further Natterer's bats in France and Germany, respectively, tested BBLV positive in 2012 (Picard-Meyer et al., 2013, Freuling et al., submitted). To date, these three isolations are the only reported rabies cases in Natterer's bats.

Antigenic patterns in reactions with anti-nucleocapsid monoclonal antibodies clearly distinguish BBLV from other lyssaviruses (Schneider et al., 1985; Annex, Table 1). This difference was supported by antigenic cartography (Horton et al., 2010) where BBLV was separated from all characterized lyssaviruses, but related to phylogroup I viruses. In this test, BBLV was antigenically equidistant from RABV and EBLV-2 (average 3.9 and 4.3AU respectively) but further from EBLV-1 viruses (average 6.5 AU). Furthermore, a comparative study with a panel of sera from human RABV vaccinees (for details of protocol see Malerzcyk et al., 2007) showed cross-neutralization with BBLV, albeit with the lowest level of correlation (Annex, Figure 3), supporting that BBLV is a separate member of phylogroup 1 lyssaviruses.

The negative-sense RNA genome of BBLV is 11,900 nt long, encoding for 5 genes: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-

dependent RNA polymerase (L). Thus, the genome organization and sequence relationships are consistent with the classification as a lyssavirus. Using concatenated N-P-M-G-L gene sequences all BBLV sequences demonstrate 80% and 79% identity to the sequence of the most similar viruses KHUV and EBLV-2, respectively, while the intra-species identity values ranged between 92.7% and 98.6%. Phylogenetic analysis, performed on different genome fragments, demonstrated limited relatedness between BBLV and all other lyssavirus species (Annex, Figure 2). Specifically, although BBLV is related to EBLV-2 and KHUV it cannot be included in these species (Annex, Figure 2).

Therefore, BBLV meets the criteria for a novel lyssavirus species based on genetic distance, phylogenetic reconstructions, antigenic patterns and ecologic features (Dietzgen et al., 2011).

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Badrane H, Bahloul C, Perrin P, Tordo N (2001) Evidence of two Lyssavirus phylogroups with distinct pathogenicity and immunogenicity. J Virol 75: 3268-3276.

Bourhy H, Kissi B, Tordo N (1993) Molecular diversity of the Lyssavirus genus. Virology 194: 70-81.

Dietzgen, R. G., Calisher, C. H., Kurath, G., Kuzmin, I. V., Rodriguez, L. L., Stone, D. M., Tesh, R. B., Tordo, N., Walker, P. J., Wetzel, T. and Whitfield, A. E. (2011). Rhabdoviridae. In Andrew M. Q. King, Michael J. Adams, Eric B. Carstens and Elliot J. Lefkowitz (Ed.), *Virus taxonomy: Ninth report of the International Committee on Taxonomy of Viruses* (pp. 654-681) Oxford, United Kingdom: Elsevier.

Freuling CM, Beer M, Conraths FJ, Finke S, Hoffmann B, et al. (2011) Novel lyssavirus in Natterer's bat, Germany. Emerg Infect Dis 17: 1519-1522.

Freuling, C.M., Abendroth, B., Beer, M., Fischer, M., Hanke, D., Hoffmann, B., Höper, D. Just, F. , Mettenleiter, T.C., Schatz, J., Müller, T: Molecular diagnostics for the detection of Bokeloh bat lyssavirus in a bat from Bavaria, Germany, Virus research submitted

Horton DL, McElhinney LM, Marston DA, Wood JL, Russell CA, et al. (2010) Quantifying antigenic relationships among the lyssaviruses. J Virol 84: 11841-11848.

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Picard-Meyer, E., Servat, A., Robardet, E., Moinet, M., Borel, C. & Cliquet, F. (2013). Isolation of Bokeloh bat lyssavirus in Myotis nattereri in France. Arch Virol, 1-8.

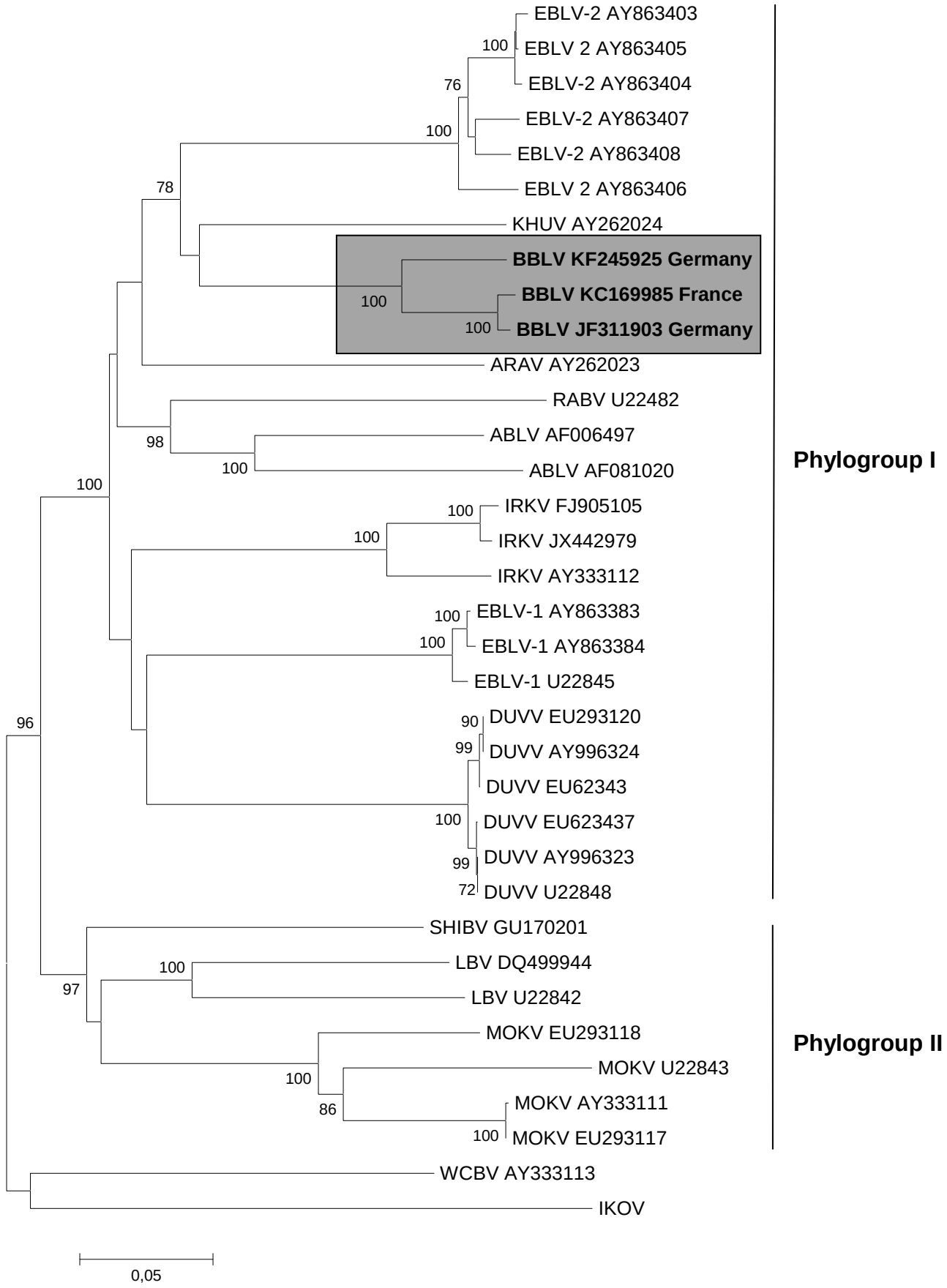
Schatz, J., Fooks, A. R., McElhinney, L., Horton, D., Echevarria, J., Vazquez-Moron, S., Kooi, E. A., Rasmussen, T. B., Muller, T. & Freuling, C. M. (2013). Bat Rabies Surveillance in Europe. Zoonoses Public Health 60, 22-34.

Schneider, L. G., Barnard, B. J. H., Schneider, H. P., Odegaard, O. A., Mueller, J., Selimov, M., Cox, J. H., Wandeler, A. I., Blancou, J. & Meyer, S. (1985). Application of monoclonal antibodies for epidemiological investigations and oral vaccination studies. In Rabies in the tropics, pp. 47-59. Edited by E. Kuwert & C. Merieux. Berlin: Springer Verlag.

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.

A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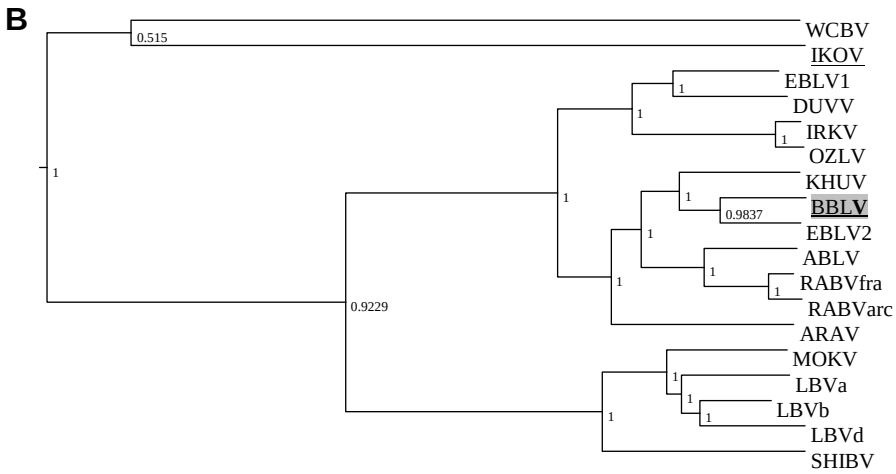
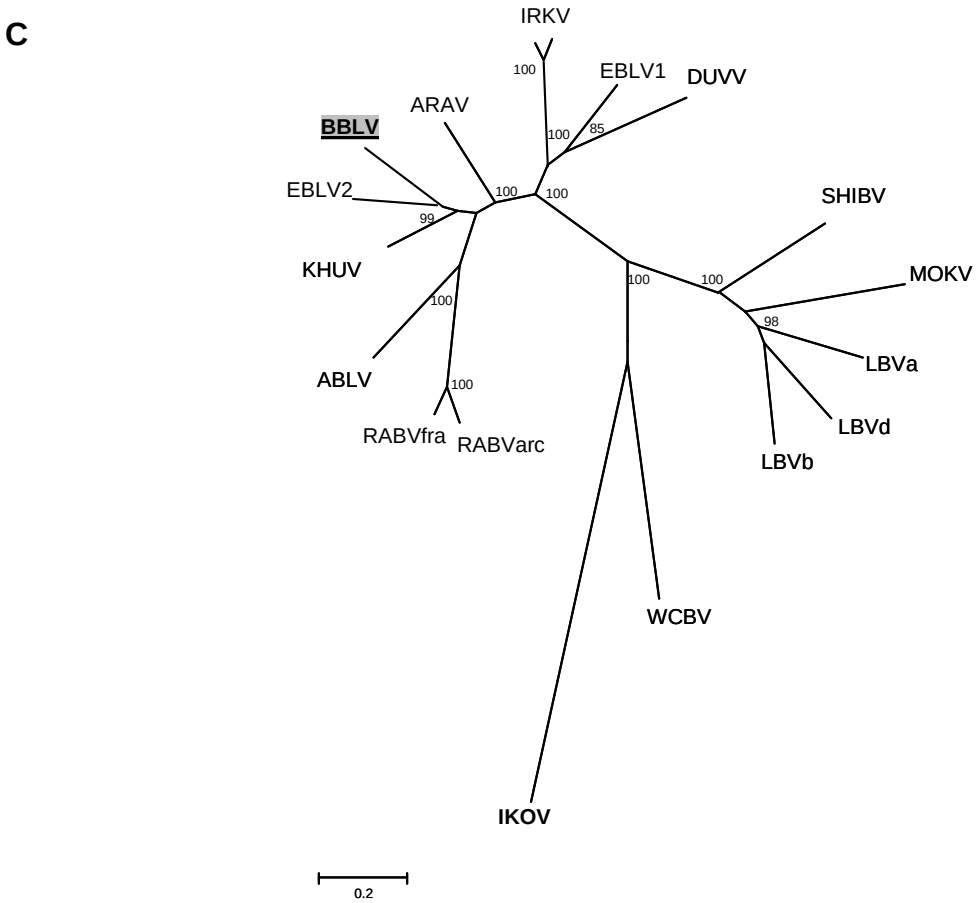
EBLV 2 AY863405
EBLV-2 AY863404
EBLV-2 AY863407
EBLV-2 AY863408
EBLV 2 AY863406
KHUV AY262024
BBLV KF245925 Germany
BBLV KC169985 France
BBLV JF311903 Germany
ARAV AY262023
RABV U22482
ABLV AF006497
ABLV AF081020
IRKV FJ905105
IRKV JX442979
IRKV AY333112
EBLV-1 AY863383
EBLV-1 AY863384
EBLV-1 U22845
DUVV EU293120
DUVV AY996324
DUVV EU62343
DUVV EU623437
DUVV AY996323
DUVV U22848
SHIBV GU170201
LBV DQ499944
LBV U22842
MOKV EU293118
MOKV U22843
MOKV AY333111
MOKV EU293117
WCBV AY333113
IKOV
0,05
Phylogroup I
Phylogroup II





Annex Figure 2. Phylogenetic reconstructions of the *Lyssavirus* genus based on full N-gene sequences with neighbour-joining (A), bayesian (B), or full genome sequence with maximum likelihood (C) evolutionary models. Significant bootstrap values or posterior probabilities are shown for key nodes.

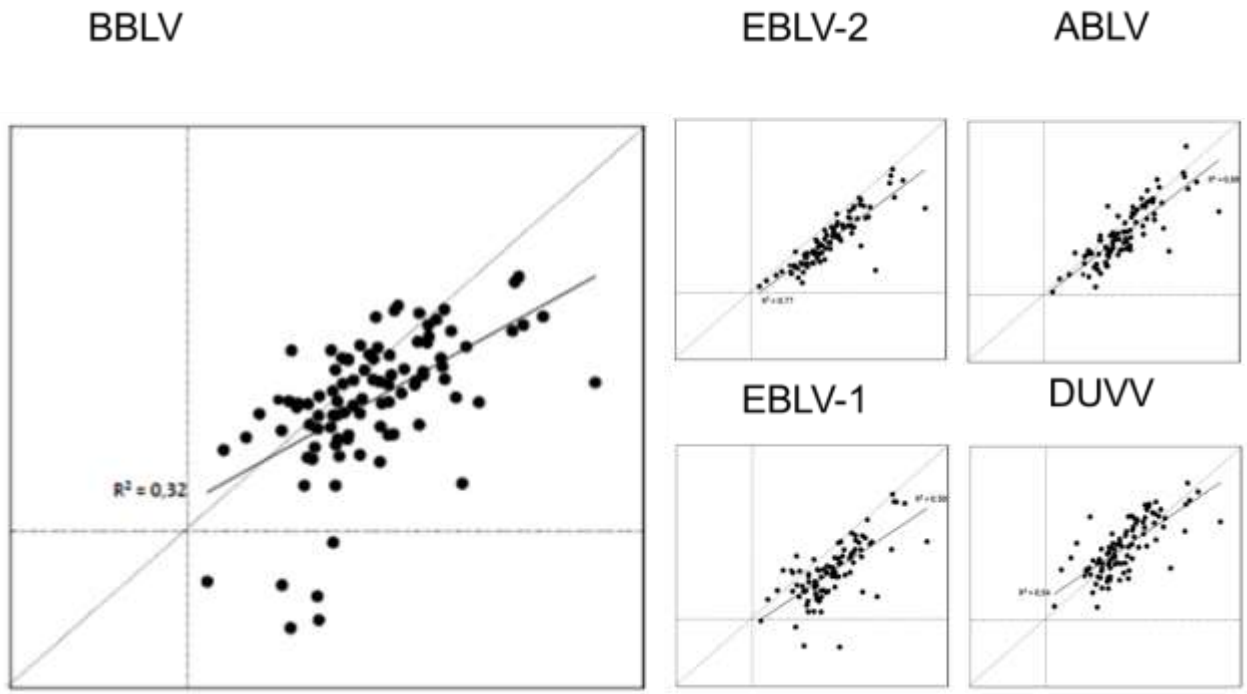


Figure 3: Results of a cross-neutralization study using a panel of human sera after vaccination with PCECV. Virus neutralizing antibody (VNA) concentrations against CVS-11 and other non-RABV lyssaviruses were determined by using a modified rapid fluorescent focus inhibition test (RFFIT).

Table 1: Reaction pattern of a panel of 10 anti-nucleocapsid monoclonal antibodies with selected lyssaviruses including **BBLV** as described before (Schneider et al., 1985).

anti-NC mAb	RABV	LBV	MOKV	DUVV	EBLV-1	EBLV-2	ABLV	**BBLV**
W239.17	+++	+++	+++	+++	+++	+++	+++	**+++**
W187.5	+++	-	-	-	-	-	+++	**-**
W187.11.2	+++	-	-	-	-	-	+++	**+++**
MW187.6.1	+++	+++	+++	+++	-	-	+++	**+++**
MSA6.3	-	-	+++	-	+++	+++	-	**+++**
LBV7.36	-	+++	-	-	-	+++	-	**-**
DUV6.15.19	-	-	-	+++	+++	-	-	**-**
S62.1.2	-	-	-	-	+++	+++	-	**-**
P 41	-	-	-	-	-	-	-	**-**
Z144.88	-	-	-	-	-	-	-	**-**

Table 2: Nucleotide identity values for concatenated coding regions (N, P, M, G and L genes) of BBLV in comparison with lyssaviruses from all identified species. Full genomes of BBLV (JF311903), RABV (M31046; EU293111; EU293115; EU293113; EU293116), DUVV (EU293120; EU293119), EBLV-1 (EU293109, EU293112, EF157976), IKOV (JX193798), ABLV (NC_003243; AF081020), KHUV (EF614261), IRKV (FJ905105, EF614260), EBLV-2 (EF157977, EU293114), ARAV (EF614259), LBV (EU293108; EU293110), MOKV (EU293118, EU293117), SHIBV (GU170201) and WCBV (EF614258) were derived from NCBI Genbank. Concatenated sequences were aligned using ClustalW and a distance matrix was calculated as implemented in BioEdit.

Species	RABV	LBV	MOKV	DUVV	EBLV-1	EBLV-2	ABLV	ARAV	KHUV	IRKV	WCBV	SHIBV	**BBLV**	**IKOV**
RABV	81.6-92.3													
LBV	67.0-67.7	76.2												
MOKV	66.9-67.3	73.3-74.1	86.6											
DUVV	71.2-71.8	67.4-67.6	67.0-67.1	98.9										
EBVL-1	71.7-72.4	68.1-68.5	67.3-67.7	76.1	95.6-98.1									
EBLV-2	72.7-73.8	67.3-68.1	67.8-68.0	73.1-73.3	74.2-74.4	98.2								
ABLV	73.2-73.8	67.0-67.4	66.4-66.8	71.2	72.2-72.3	73.9								
ARAV	72.9-73.2	68.2-68.3	67.7-68.1	73.4-73.5	75.4-75.5	76.9	73.6							
KHUV	72.9-73.4	67.5-68.0	67.1-67.3	73.5-73.6	74.7	78.7-78.9	74.5	77.5						
IRKV	71.5-72.3	67.9-68.5	67.7-68.3	74.3-74.4	76.3-76.5	73.9	71.6	73.6-74.2	73.6-74.3	91.9				
WCBV	64.8-65.5	65.8-66.0	65.3-65.5	65.8	65.5-65.7	65.5	65.2	65.7	65.4	65.2				
SHIBV	67.2-67.7	73.8-75.1	71.9-72.0	67.7-67.8	68.2-68.3	68.1	67.2	68.1	67.9	68.7	66.4			
BBLV	**72.7-73.6**	**67.5-67.9**	**67.6-68.1**	**73.1**	**74.2-74.3**	**78.2**	**74.3**	**76.3**	**78.4**	**73.6**	**65.1**	**68.7**		
IKOV	61.9-62.5	62.7-62.9	62.3-62.5	62.5-62.6	62.6-62.7	62.8	62.3	62.6	62.4	62.4	63.2	63.5	62.5	

Table 3: The lengths of coding and non-coding regions of lyssavirus genomes.

	RABV	LBV	MOKV	DUVV	EBLV-1	EBLV-2	ABLV	ARAV	KHUV	IRKV	WCBV	SHIBV	**BBLV**	IKOV
3' UTR*	70	70	70	70	70	70	70	70	70	70	70	70	70	70
N protein	**1353**	**1353**	**1353**	**1356**	**1356**	**1356**	**1353**	**1356**	**1356**	**1356**	**1353**	**1353**	**1356**	1353
N–P	90-1	101	100-102	90	90	101	94	85	95	93	64	98	91	66
P protein	**894**	**918**	**912**	**897**	**897**	**894**	**894**	**894**	**894**	**897**	**894**	**918**	**894**	870
P–M	88	75	80	83	83	88	89	85	72	82	133	76	86	74
M protein	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	**609**	609
M–G	211-5	204	203-204	191	211	210 (205)	207-209	210	208	214	206	205	210	209
G protein	**1575**	**1569**	**1569**	**1602**	**1575**	**1575**	**1578-1581**	**1581**	**1581**	**1575**	**1578**	**1569**	**1575**	1575
G–L	522	578-588	546-563	562-563	560	512	508-509	514	504	569	862	613	496	569
L protein	**6384***	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	**6384**	6381
5' UTR	131	145	112-114	130-131	131	131	131	130	130	131	125	150	129	126
Genome	**11 923-8**	**12006-16**	**11 940-57**	**11 975-6**	**11 966**	**11 930**	**11 918**	**11918**	**11903**	**11980**	**12278**	**12045**	**11900**	11902

*6387 in SHBRV