# Supporting Information

## Grybchuk et al. 10.1073/pnas.1717806115

### SI Methods

**Cultivation of Trypanosomatids.** Axenic cultures of monoxenous trypanosomatids of the genera *Leptomonas*, *Crithidia*, and *Phytomonas* were obtained from (*i*) Life Science Research Centre, University of Ostrava, Ostrava, Czech Republic; (*ii*) Department of Parasitology, Charles University, Prague; (*iii*) Institute of Parasitology, Budweis, Czech Republic; (*iv*) Fundação Oswaldo Cruz, Rio de Janeiro; and (*v*) the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia. Trypanosomatids were cultivated in the Brain Heart infusion medium supplemented with 10 μg/mL of hemin, 500 units/mL of penicillin, and 0.5 mg/mL of streptomycin as described previously (105) and grown to the late logarithmic stage ($10^7$–$10^8$ cells/mL).

**Phylogenetic Analyses of Viruses.** RDRP sequences of the viruses characterized in this work were aligned with the related sequences from GenBank in the online version of MAFFT 7 using the E-INS-i method (106). Ambiguous parts of the alignments were removed with the use of TrimAl v. 1.3 (107). Positions with more than 50% gaps were filtered out by Gap Strip/Squeeze V. 2.1.0 (https://www.hiv.lanl.gov/content/sequence/GAPSTREEZE/gap.html). The resulting alignments had the following lengths: 412 aa (the *Narnaviridae* dataset), 273 aa (the *Tombus-/Nodaviridae* dataset), and 1,415 aa (the *Bunyaviridae* dataset).

Maximum likelihood phylogenetic inference was performed in IQ-TREE v. 1.4.2 (108) with automatic selection of the best-fit amino acid substitution and site heterogeneity models (four gamma categories). The best-fitted model parameters defined by Bayesian Information Criterion were LG + F + I + G for the *Narnaviridae* and *Bunyaviridae* datasets and Blosum62 + I + G4 for the *Tombus-/Nodaviridae* dataset. Gaps were treated as missing data. Edge support was estimated with a bootstrap test (1,000 standard replicates). Bayesian inference was accomplished in MrBayes 3.2.6 (109) with the analyses ran for 1 million generations (given the observed fast convergence) and trees sampled every 100 generations. The mixed amino acid substitution model was used (resulting in 1.0 posterior probability of Blosum62 for all three datasets) with the heterogeneity over sites estimated using the G + I model. Amino acid frequencies were fixed to empirical values in the *Narnaviridae* and *Bunyaviridae* datasets and were estimated from the data matrix in the *Tombus-/Nodaviridae* dataset in accordance with the best-fit model defined by IQ-TREE v. 1.4.2 for each dataset. Other parameters were left in their default states.

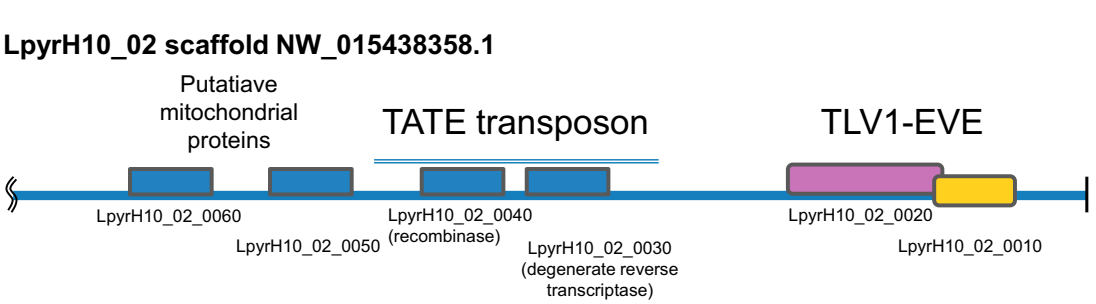
**Phylogeny of Trypanosomatid Hosts.** The core alignments of 18S rRNA and gGAPDH genes were taken from previous work (76). Ambiguously aligned positions of 18S rRNA gene alignment were removed using Gblocks 0.91b as described previously (110). The concatenated alignments were subjected to maximum likelihood analysis in IQ-TREE (108) with partitioning by gene as well as by codon position for the gGAPDH gene. The best partitioned model of nucleotide substitutions (K3Pu + G4, TIM3 + I + G4, and TPM2u + G4 for the first, second, and third codon positions of gGAPDH gene, respectively and TNe + I + G4 for 18S rRNA gene) was selected with the use of ModelFinder (111). The statistical support of branches was estimated using 1,000 replicates of the standard bootstrap method.

**Genomic and Transcriptomic Analyses.** To find trypanosomatid signature sequences in the Sequence Reads Archive (SRA, https://www.ncbi.nlm.nih.gov/sra) and transcriptome shotgun assemblies (TSAs), TBLASTN and BLASTN searches were performed with amino acid (PFR1) and nucleotide (18S rRNA) sequences, respectively. In case of SRA-blast, the retrieved reads were assembled into contigs using the CAP3 sequence assembly program with the following parameters: minimal overlap length, 20 bp; minimum identity, 100% (112). Obtained contigs were extended by successive rounds of BLASTN searches against the original SRA. Final full-length contigs as well as hits from TSAs were subjected to blast search (megablast for nucleotide sequences and blastp for translated protein-coding sequences) against the nonredundant nucleotide collection of the NCBI database for identification. A similar approach was used to search of reads corresponding to nucleocapsid proteins in SRAs, which contained RDRPs closely related to LBVs. Obtained amino acid sequences were aligned with newly identified nucleocapsid proteins of viruses characterized in this work using BLAST pairwise alignment to confirm their identities.

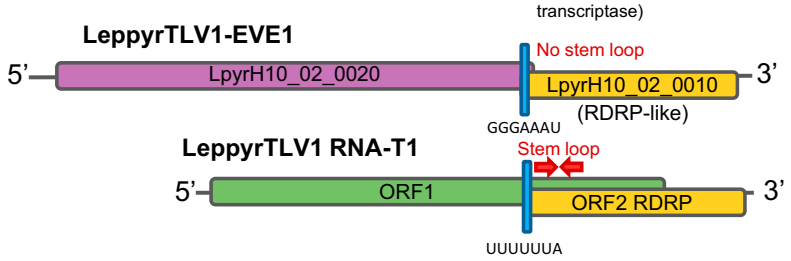
**Codon Usage Analysis in *Leptomonas pyrrhocoris* Viruses.** Codon frequencies in the protein-coding regions of the six viral genes were analyzed using the CODONW program (codonw.sourceforge.net).

**Negative-Stain Transmission Electron Microscopy.** In brief, gradient-purified virus samples were applied to a carbon-coated copper grid, stained with molybdenum acetate, and examined under a Philips 201C transmission electron microscope. The morphologies and sizes of the virus particles were analyzed as described previously (113).

A




B




C


D

| MOTIFS | | $F_1$ | | | $F_2$ | | | A | |
|---|---|---|---|---|---|---|---|---|---|
| Picorna | | | | | | | | | |
| 1RDR_A_Poliovirus | (155) | VTYVKDELRS | (164) | (169) | EQGKSRLIEASSLND | (183) | (228) | --KLFAFDYTGYDASLSP | (243) |
| P03313.4_coxsackie_virus_B3 | (156) | VTYVKDELRS | (165) | (170) | AKGKSRLIEASSLND | (184) | (230) | --HLIAFDYSGYDASLSP | (244) |
| AFK65743.1_Rhinovirus_C | (139) | ITYLKDELRS | (148) | (153) | KVGKTRAIEAASLND | (167) | (213) | --NLLVFDYTNYDGSLNP | (227) |
| P03305.1_foot-and-mouth_diseas | (160) | QTFLKDEIRP | (169) | (174) | RAGKTRIVDVLPVEH | (188) | (233) | YRNVWDVDYSAFDANHCS | (250) |
| Flavi | | | | | | | | | |
| P06935.2_west_nile_virus | (455) | NMMGKREKKP | (464) | (469) | KAKGSRAIWFMWLGA | (483) | (529) | GGKVYADDTAGWDTRITK | (546) |
| P17763.2_dengue_virus | (452) | NMMGKREKKL | (461) | (466) | KAKGSRAIWYMWLGA | (480) | (526) | GGNMYADDTAGWDTRITE | (543) |
| P19711.2_bovine_viral_diarrhea | (259) | TAIPKNEKRD | (268) | (280) | VEKRPRVIQYPEAKT | (294) | (338) | EPVAVSFDTKAWDTQVTS | (355) |
| Calici | | | | | | | | | |
| Q83883.1_Norwalk_virus | (143) | TAALKDELVK | (152) | (158) | QKVKKRLLWGADLGT | (172) | (216) | YKNHFDADYTAWDSTQNR | (233) |
| P27410.1_Rabbit_hemorrhagic_di | (142) | ACGLKDELRP | (151) | (156) | KEGKKRLLWGCDVGV | (170) | (216) | ASDFLCLDYSKWDSTMSP | (233) |
| Q6XDK8.1_Sapporo_virus | (315) | KLALKDELRP | (324) | (329) | AQGKRRLLWGCDAGA | (343) | (387) | GGVLYCLDYSKWDSTQHP | (404) |
| LeppyrTLV1-EVE1 | (158) | -SFIKDEGYD | (166) | (167) | EYKMPRAINSYSDST | (181) | (221) | DSKVLYTDFSHFESHHRG | (238) |
| LeppyrTLV1 | (157) | DSFIKDESYD | (166) | (167) | ELKAPRSIMSYPDDV | (181) | (221) | GGKVGSTDFSSYECHHSG | (238) |

| MOTIFS | | B | | | C | | | D | |
|---|---|---|---|---|---|---|---|---|---|
| Picorna | | | | | | | | | |
| 1RDR_A_Poliovirus | (286) | MPSGCSGTSIFNSMINNLII | (305) | (322) | KMIAYGDDVIASYP | (335) | (348) | KDYGLTM---T | (355) |
| P03313.4_coxsackie_virus_B3 | (288) | MPSGCSGTSIFNSMINNIII | (307) | (324) | RMIAYGDDVIASYP | (337) | (350) | KGYGLIM---T | (357) |
| AFK65743.1_Rhinovirus_C | (268) | MPSGISGTSIFNTIINNIII | (287) | (304) | KIVAYGDDVIASYP | (317) | (330) | VKYGLTI---T | (337) |
| P03305.1_foot-and-mouth_diseas | (296) | MPSGCSATSIINTILNNIYV | (315) | (332) | TMISYGDDIVVASD | (345) | (358) | KSLGQTI---T | (365) |
| Flavi | | | | | | | | | |
| P06935.2_west_nile_virus | (602) | RGSGQVVTYALNTFTNLAVQ | (621) | (662) | RMAVSGDDCVVKP- | (674) | (687) | NAMSKVRKDIQ | (697) |
| P17763.2_dengue_virus | (598) | RGSGQVGTYGLNTFTNMEAQ | (617) | (656) | RMAISGDDCVVKP- | (668) | (681) | NDMGKVRKDIP | (691) |
| P19711.2_bovine_viral_diarrhea | (403) | RGSGQPDTSAGNSMLNVLTM | (422) | (442) | RIHVCGDDGFLITE | (455) | (471) | HEAGKPQK-IT | (480) |
| Calici | | | | | | | | | |
| Q83883.1_Norwalk_virus | (279) | LPSGFPCTSQVNSINHWIIT | (298) | (318) | YFSFYGDDEIVSTD | (331) | (344) | KEYGLKP---T | (351) |
| P27410.1_Rabbit_hemorrhagic_di | (279) | LPSGMPFTSVINSICHWLLW | (298) | (321) | PFYTYGDDGVYAMT | (334) | (349) | RDYGLSP---T | (356) |
| Q6XDK8.1_Sapporo_virus | (450) | LPSGMPFTSVINSLNHMTYF | (469) | (493) | TVHTYGDDCLYSVC | (506) | (521) | TSFGLKP---T | (528) |
| LeppyrTLV1-EVE1 | (284) | LMSGALWTSFQNSFLNFFVM | (303) | (323) | KMFIEGDDGIMKHF | (336) | (344) | KRLGLCLK-IN | (353) |
| LeppyrTLV1 | (288) | LMSGALWTSFQNSFLNLMMI | (307) | (325) | DTFIEGDDGIFRAF | (338) | (346) | ARLGIKLK-IS | (355) |

**Fig. S1.** LeppyrTLV1 EVE1. (*A*) The endogenous viral element is located at the subtelomeric region of the *Leptomonas pyrrhocoris* chromosome (scaffold NW_015438358.1). The EVE1 element is preceded by the reverse transcriptase-coding TATE DNA transposon indicating the possible mechanism for the LeppyrTLV1 endogenization. (*B*) Comparison of the ORF organization of RDRP-coding RNA-T1 of 1 LeppyrTLV1 and its endogenous viral element. (*C*) The overlap region between the ORFs of EVE1 contains a putative slippery sequence (underlined) capable of driving a −1 ribosomal frameshift, similarly to LeppyrTLV1. (*D*) Multiple alignments of RDRPs of LeppyrTLV1 and LeppyrTLV1-EVE1 with RNA polymerases of *Picorna*-, *Flavi*-, and *Caliciviridae*. Identical residues are indicated in red; similar residues are in blue. Each block shows amino acid motifs typically found in viral RNA polymerases (59).

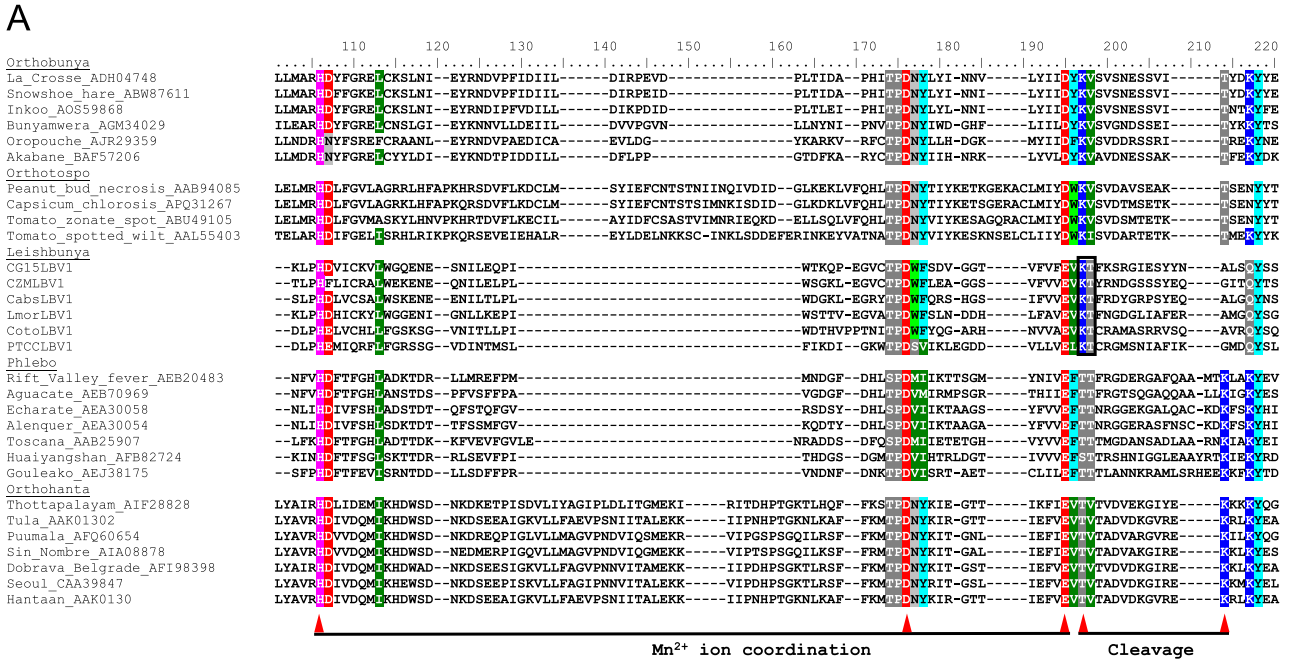



**Fig. S2.** Leishbunyavirus protein domains. (*A*) Amino acid alignment of the N-terminal endonuclease domain of the L protein of leishbunyaviruses and other bunyaviruses. Functionally important residues are marked with arrowheads. Residues implicated in nucleolytic cleavage in leishbunyaviruses are boxed. (*B*) Amino acid alignment of nucleocapsid proteins of leishbunyaviruses reported in ref. 34 and this work (TrypLBVs) and assembled from metatranscriptomes (metatranscriptomic BVs). *Cumuto* and *Gouleako* viruses are shown for comparison. Indels are shown as dashes. Positions with more than 82% similarity are shaded; those with 90% similarity are starred.

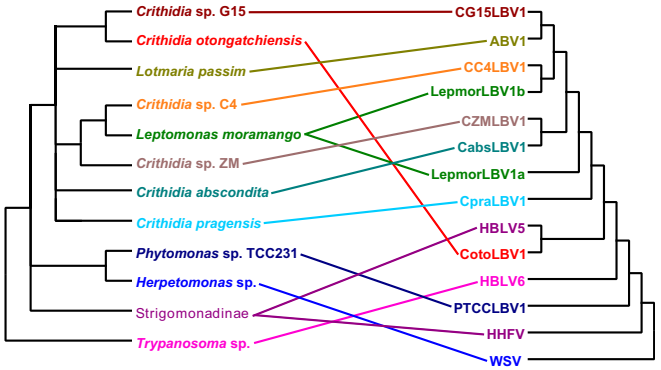



**Fig. S3.** Comparison of the phylogenies of leishbunyaviruses and their respective hosts. The scheme is based on the phylogenetic trees presented in Figs. 3 and 5 with simplifications: (*i*) no branch lengths are shown on either tree; (*ii*) only LBVs and LBV-containing trypanosomatids were included; and (*iii*) all clades with bootstrap support below 70% were collapsed and reshuffled to minimize the number of potential viral transitions between hosts.

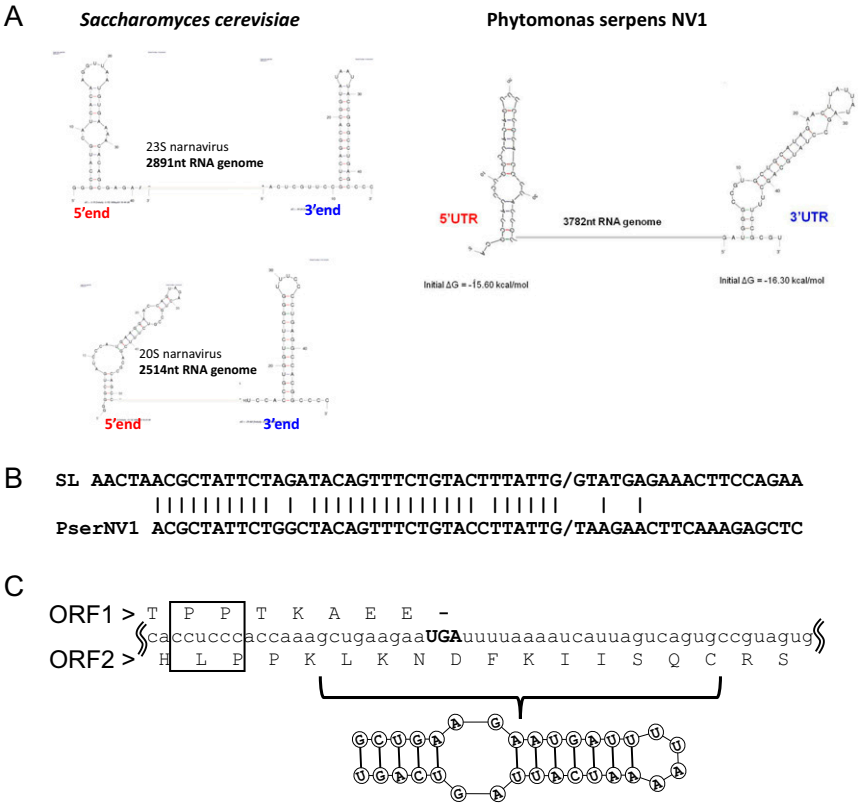



**Fig. S4.** Features of genomic RNAs of PserNV1 and LepseyNLV1. (*A*) Comparison of terminal complementary sequences and stem-loop structures with those of narnaviruses from *Saccharomyces cerevisiae*. (*B*) Nucleotide alignment of the 5′ end of PserNV1 and the SL RNA gene from *Phytomonas serpens*. The SL/miniexon boundary is indicated by a slash symbol. (*C*) Features of a potential frameshift region between ORF1 and ORF2 of the LepseyNLV1 small segment. The location of a hairpin motif is marked by a bracket, which has a predicted ΔG of −6 kCal [OligoAnalyzer 3.1 (114)]. A putative pyrimidine-rich slippery sequence (CCUCCC) positioned 6 nt upstream is shown in the box.

**Table S1. Complete list and properties of the studied trypanosomatid species**

| Species | Isolate | Reference to trypanosomatid description | Host | Geographic origin | Presence of viruses |
|---|---|---|---|---|---|
| *Crithidia abscondita* | 127AL | Yurchenko et al. (2009) (1) | *Largus* sp. | Ecuador | *Bunyavirales* |
| *Crithidia brachyflagelli* | 340VL | Jirků et al. (2012) (2) | *Prepos cf accinctus* | Costa Rica | Not observed |
| *Crithidia brevicula* | 101 | Kostygov et al. (2014) (3) | *Nabis flavomarginatus* | Russia | Not observed |
| *Crithidia brevicula* | KV1 | Kostygov et al. (2014) (3) | *Gerris lacustris* | Russia | Not observed |
| *Crithidia brevicula* | F6 | Kostygov et al. (2014) (3) | *Nabis flavomarginatus* | Russia | Not observed |
| *Crithidia fasciculata* | Finn-01.02 | Hamilton et al. (2015) (4) | *Drosophila falleni* | USA | Not observed |
| *Crithidia fasciculata* | COLPROT053 | Wallace and Clark (1959) (5) | *Phaenicia sericata* | USA | Not observed |
| *Crithidia fasciculata* | COLPROT048 | Wallace (1943) (6) | *Anopheles quadrimaculatus* | France | Not observed |
| *Crithidia otongatchiensis* | Ecu-08 | Yurchenko et al. (2014) (7) | Family Syrphidae | Ecuador | *Bunyavirales* |
| *Crithidia permixta* | 128SI | Yurchenko et al. (2009) (1) | Tribe Mirini | Ecuador | Not observed |
| *Crithidia pragensis* | MCZ-11 | Yurchenko et al. (2014) (7) | *Cordilura albipes* | Czech Republic | *Bunyavirales* |
| *Crithidia* sp. | G15 | Votýpka et al. (2012b) (8) | *Rhynocoris rapax* | Ghana | *Bunyavirales* |
| *Crithidia* sp. | ZM | Podlipaev and Frolov (1987) (9) | *Lygocoris lucorum* | Russia | *Bunyavirales* |
| *Crithidia* sp. | C4 | Podlipaev et al. (2004) (10) | *Limnoporus rufoscutellatus* | Russia | *Bunyavirales* |
| *Crithidia* sp. | Cfm9 | Merzlyak et al. (2001) (11) | *Nabis flavomarginatus* | Russia | Not observed |
| *Crithidia* sp. | CL6 | Podlipaev and Rokitskaya (1999) (12) | *Nabis limbatus* | Russia | Not observed |
| *Crithidia thermophila* | 320AR | Jirků et al. (2012) (2) | *Largus maculatus* | Costa Rica | Not observed |
| *Crithidia thermophila* | COLPROT018 | Roitman et al. (1977) (13) | *Cosmoclopius* sp. | Brazil | Not observed |
| *Crithidia thermophila* | COLPROT054 | Roitman et al. (1977) (13) | *Zelus leucogrammus* | Brazil | Not observed |
| *Zelonia costaricensis* | 15EC | Yurchenko et al. (2006b) (14) | *Ricolla simillima* | Costa Rica | Not observed |
| *Leptomonas jaderae* | 34EC | Yurchenko et al. (2009) (1) | *Jadera obscura* | Costa Rica | Not observed |
| *Leptomonas moramango* | MMO-09 | Yurchenko et al. (2014) (7) | *Pachycerina cf vaga* | Madagascar | *Bunyavirales* |
| *Leptomonas pyrrhocoris* | F165 | Votýpka et al. (2012a) (15) | *Pyrrhocoris apterus* | France | LeppyrTLV1 |
| *Leptomonas pyrrhocoris* | F19 | Votýpka et al. (2012a) (15) | *Pyrrhocoris apterus* | France | LeppyrTLV1, LeppyrOV1 |
| *Leptomonas pyrrhocoris* | H10 | Votýpka et al. (2012a) (15) | *Pyrrhocoris apterus* | Czech Republic | LeppyrTLV1, LeppyrOV1, LeppyrTLV1-EVE |
| *Leptomonas pyrrhocoris* | 10VL | Votýpka et al. (2012a) (15) | *Dysdercus obliquus* | Costa Rica | Not observed |
| *Leptomonas pyrrhocoris* | 121AL | Votýpka et al. (2012a) (15) | *Dysdercus obscuratus* | Ecuador | Not observed |
| *Leptomonas pyrrhocoris* | 122AL | Votýpka et al. (2012a) (15) | *Dysdercus obscuratus* | Ecuador | Not observed |
| *Leptomonas pyrrhocoris* | 14BT | Votýpka et al. (2012a) (15) | *Dysdercus lunulatus* | Costa Rica | Not observed |
| *Leptomonas pyrrhocoris* | 25EC | Votýpka et al. (2012a) (15) | *Dysdercus* sp. | Costa Rica | Not observed |
| *Leptomonas pyrrhocoris* | 28EC | Votýpka et al. (2012a) (15) | *Dysdercus* sp. | Costa Rica | Not observed |
| *Leptomonas pyrrhocoris* | 324RV | Votýpka et al. (2012a) (15) | *Dysdercus obscuratus* | Costa Rica | Not observed |
| *Leptomonas pyrrhocoris* | 329MV | Votýpka et al. (2012a) (15) | *Dysdercus mimulis* | Costa Rica | Not observed |
| *Leptomonas pyrrhocoris* | CH278 | Votýpka et al. (2012a) (15) | *Dysdercus poecilus* | China | Not observed |
| *Leptomonas pyrrhocoris* | G58 | Votýpka et al. (2012a) (15) | *Dysdercus fasciatus* | Ghana | Not observed |
| *Leptomonas pyrrhocoris* | K06 | Votýpka et al. (2012a) (15) | *Scantius aegyptius* | Cyprus | Not observed |
| *Leptomonas pyrrhocoris* | P59 | Votýpka et al. (2012a) (15) | *Pyrrhocoris marginatus* | Czech Republic | Not observed (LeppyrTLV1-EVE) |
| *Leptomonas pyrrhocoris* | SERG | Flegontov et al. (2016) (16) | *Dysdercus* sp. | Burkina Faso | Not observed |
| *Leptomonas pyrrhocoris* | PP1 | Frolov et al. (2014) (17) | *Pyrrhocoris apterus* | Russia | Not observed (LeppyrTLV1-EVE) |
| *Leptomonas pyrrhocoris* | PP2 | Frolov et al. (2014) (17) | *Pyrrhocoris apterus* | Russia | Not Observed (LeppyrTLV1-EVE) |
| *Leptomonas pyrrhocoris* | LP | Yurchenko et al. (2006a) (18) | *Pyrrhocoris apterus* | Russia | Not Observed (LeppyrTLV1-EVE) |
| *Leptomonas scantii* | F221 | Votýpka et al. (2012a) (15) | *Scantius aegyptius* | France | Not observed |
| *Leptomonas seymouri* | ATCC | Wallace (1977) (19) | *Dysdercus suturellus* | USA | *Narnaviridae* |
| *Leptomonas tarcoles* | 47VL | Yurchenko et al. (2008) (20) | *Prepops* sp. | Costa Rica | Not observed |
| *Phytomonas serpens* | 9T (CB) | Jankevicius et al. (1989) | *Lycopersicon esculentum* | Brazil | Not observed |
| *Phytomonas serpens* | 9T (UCR) | Jankevicius et al. (1989) | *Lycopersicon esculentum* | Brazil | *Narnaviridae* |

**Table S1. Cont.**

| Species | Isolate | Reference to trypanosomatid description | Host | Geographic origin | Presence of viruses |
|---|---|---|---|---|---|
| *Phytomonas serpens* | 30T | Jankevicius et al. (1989) (21) | *Lycopersicon esculentum* | Brazil | *Narnaviridae* |
| *Phytomonas serpens* | 1G | Da Silva and Roitman (1990) (22) | *Phthia picta* | Brazil | Not observed |
| *Phytomonas serpens* | 15T, COLPROT186 | Jankevicius et al. (1989) (21) | *Solanum lycopersicum* | Brazil | Not observed |
| *Phytomonas* sp. | TCC231 | Jankevicius et al. (1989) (21) | *Lycopersicon esculentum* | Brazil | Bunyavirales |
| *Phytomonas* sp. | COLPROT079 | Kastelein and Parsadi (1988) (23) | *Allamanda cathatica* | Suriname | Not observed |
| *Phytomonas* sp. | COLPROT080 | Conchon et al. (1989) (24) | *Citrus bergamia* | Brazil | Not observed |


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**Table S2. Sequences of viral RDRPs with working abbreviations of viral names used in phylogenetic inferences**

| Clade | Short name | Virus name | Accession no. | Host |
|---|---|---|---|---|
| *Tombus*-like virus from *Leptomonas pyrrhocoris* (Fig. 1*D*) | | | | |
| *Tombusviridae* | JINRV | *Japanese iris necrotic ring virus* | BAA92792.1 | Plants (*Iris ensata*) |
| *Tombusviridae* | TCV | *Turnip crinkle virus* | AAP78486.1 | Plants |
| *Tombusviridae* | MNSV | *Melon necrotic spot virus* | ABC67516.1 | Plants |
| *Tombusviridae* | CarMV | *Carnation mottle virus* | CAB38331.1 | Plants |
| *Tombusviridae* | OMMV | *Olive mild mosaic virus* | AEC50092.1 | Plants (*Olea europaea*) |
| *Tombusviridae* | PoLV | *Pothos latent virus* | Q9IWA0.1 | Plants |
| *Tombusviridae* | TBSV | *Tomato bushy stunt virus* | AAT67237.1 | Plants |
| *Tombusviridae* | CTLV14 | *Changjiang tombus-like virus 14* | APG76248.1 | Crustacea: *Procambarus clarkia* |
| *Tombus*-like viruses | HTLV13 | *Hubei tombus-like virus 13* | APG76577.1 | Myriapoda: Scutigeridae |
| *Tombus*-like viruses | STLV6 | *Sanxia tombus-like virus 6* | APG76428.1 | Insecta: *Gerridae* sp. |
| *Tombus*-like viruses | BTLV9 | *Beihai tombus-like virus 9* | APG76191.1 | Mollusca: Octopodidae sp. |
| *Tombus*-like viruses | HTLV22 | *Hubei tombus-like virus 22* | APG76327.1 | Insecta: *Paracercion melanotum*, *Paracercion calamorum*, *Ceriagrion auranticum*, *Brachydiplax chalybea*, *Orthetrum albistylum*, *Pseudothemis zonata*, *Chironomus* sp. |
| *Tombus*-like viruses | HTLV36 | *Hubei tombus-like virus 36* | APG76457.1 | Myriapoda: *Diplopoda* sp, *Otostigmus* scaber, *Scolopocryptops* sp., *Otostigmus scaber*, *Myriapoda* sp. |
| *Tombus*-like viruses | CTLV22 | *Changjiang tombus-like virus 22* | APG76278.1 | Crustacea: *Procambarus clarkia* |
| *Tombus*-like viruses | BUC1 | *Brandmavirus UC1* | AHA86931.1 | Uunknown |
| Tombus-like viruses | WTLV17 | *Wenzhou tombus-like virus 17* | APG76615.1 | Mollusca: *Pomacea canaliculata* |
| *Tombus*-like viruses | HTLV35 | *Hubei tombus-like virus 35* | APG76480.1 | Myriapoda: *Diplopoda* sp., *Otostigmus scaber*, *Scolopocryptops* sp, *Otostigmus scaber*, *Myriapoda* sp. |
| *Tombus*-like viruses | LeppyrTLV1-EVE1 | *LeppyrTLV1 endogenous virus element* | | Trypanosomatidae |
| *Tombus*-like viruses | LeppyrTLV1 | *Leptomonas pyrrhocoris tombus-like virus 1* | | Trypanosomatidae |
| *Tombus*-like viruses | BTLV17 | *Beihai tombus-like virus 17* | APG76183.1 | Mollusca: *Solen strictus* |
| *Tombus*-like viruses | BTLV18 | *Beihai tombus-like virus 18* | APG76207.1 | Mollusca: Octopodidae sp. |
| *Tombus*-like viruses | Towan | *Towan virus* | AOG30801.1 | Unknown |
| *Tombus*-like viruses | JTLV2 | *Jingmen tombus-like virus 2* | APG76305.1 | Nematoda: *Ascaridia* sp. |
| CBPV-like viruses | LSV1 | *Lake Sinai virus 1* | AEH26193.1 | Insecta: *Apis mellifera* |
| CBPV-like viruses | AaCV | *Anopheline-associated C virus* | AGW51750.1 | Insecta: *Anopheline* sp. |
| CBPV-like viruses | CBPV | *Chronic bee paralysis virus* | ACO82537.1 | Insecta: *Apis mellifera* |
| CBPV-like viruses | Dansoman | *Dansoman virus* | AKH40306.1 | Insecta: *Drosophila* sp. |
| CBPV-like viruses | HTLV42 | *Hubei tombus-like virus 42* | APG76280.1 | Insecta (Diptera): *Atherigona orientalis*, *Chrysomya megacephala*, *Lucilia sericata*, *Musca domestica*, *Sarcophaga dux*, *S. peregrine*, *Sarcophaga* sp. |
| CBPV-like viruses | BTLV19 | *Beihai tombus-like virus 19* | APG76134.1 | Crustacea: *Amphibalanus rhizophorae* |
| CBPV-like viruses | WeTLV4 | *Wenling tombus-like virus 4* | APG76579.1 | Crustacea: *Brachyura* sp., *Achelata* sp., *Penaeoidea* spp., *Ibacus novemdentatus*, *Anomura* sp, *Penaeidae* sp., *Charybdis bimaculata*, *Charybdis rufodactylus*, Latreilliidae sp., *Ovalipes punctatus* |

**Table S2. Cont.**

| Clade | Short name | Virus name | Accession no. | Host |
|---|---|---|---|---|
| CBPV-like viruses | WCV4 | *Wenzhou crab virus 4* | APG76640.1 | Crustacea: *Charybdis hellerii*, *Charybdis japonica*, *Charybdis lucifera* |
| CBPV-like viruses | WTLV18 | *Wenzhou tombus-like virus 18* | APG76097.1 | Mollusca: *Barbatia virescens*, *Sinonovacula constricta*, *Tegillarca granosa*, *Crassostrea ariakensis*, *Mytilus coruscus* |
| CBPV-like viruses | PHVA | *Plasmopara halstedii virus A* | ADK55578.1 | Oomycetes: *Plasmopara halstedii* |
| CBPV-like viruses | SmVA | *Sclerophthora macrospora virus A* | BAC11954.1 | Oomycetes: *Sclerophthora macrospora* |
| *Nodaviridae* | FHV | *Flock house virus* | CAA54399.1 | Insecta (Coleoptera): *Costelytra zealandica* |
| *Nodaviridae* | NoV | *Nodamura virus* | AAF97860.1 | Insecta (Diptera): *Culex tritaeniorhynchus*) |
| *Nodaviridae* | PaV | *Pariacoto virus* | AAF71691 | Insecta (Lepidoptera): *Spodoptera eridania* |
| *Nodaviridae* | SJNNV | *Striped Jack nervous necrosis virus* | BAB64329.1 | Chordata: *Cocinero* sp. |
| Leishbunyaviruses (Fig. 3*D*) | | | | |
| *Phlebovirus* | CDUV | *Chandiru virus* | AEA30057.1 | Arthropod vectors (Diptera), vertebrate hosts |
| *Phlebovirus* | PTV | *Punta Toro phlebovirus* | ALL45372.1 | Arthropod vectors (Diptera), vertebrate hosts |
| *Phlebovirus* | BUJV | *Bujaru virus* | API68880.1 | Arthropod vectors (Diptera), vertebrate hosts |
| *Phlebovirus* | Adana | *Adana virus* | AJK91618.1 | Arthropod vectors (Diptera), vertebrate hosts |
| *Phlebovirus* | SALV | *Salehabad phlebovirus* | AGA82741.1 | Arthropod vectors (Diptera), vertebrate hosts |
| *Phlebovirus* | SFNV | *Sandfly fever Naples virus* | CAA48478.1 | Arthropod vectors (Diptera), vertebrate hosts |
| *Phlebovirus* | RVFV | *Rift Valley fever virus* | ABD51507.1 | Arthropod vectors (Diptera), vertebrate hosts |
| | SFTSV | *Severe fever with thrombocytopenia virus* | ADZ04470.1 | Arthropod vectors (Diptera or ticks), vertebrate hosts |
| | BHAV | *Bhanja virus* | AFO66272.1 | Arthropod vectors (Diptera or ticks), vertebrate hosts |
| | LSV | *Lone Star virus* | AGL50921.1 | Arthropod vectors (Diptera or ticks), vertebrate hosts |
| *Phlebovirus* (uukuniemi group) | EgAN | *EgAN 1825–61 virus* | AEL29654.1 | Arthropod vectors (ticks), vertebrate hosts |
| *Phlebovirus* (uukuniemi group) | UU.K.V | *Uukuniemi phlebovirus* | BAA01590.1 | Arthropod vectors (ticks), vertebrate hosts |
| *Phlebovirus* (uukuniemi group) | Khasan | *Khasan virus* | AII79370.1 | Arthropod vectors (ticks), vertebrate hosts |
| *Phasivirus* | Badu | *Badu phasivirus* | AMA19446.1 | Insects (mosquitoes) |
| *Phasivirus* | WMPV | *Wutai mosquito phasivirus* | AJG39270.1 | Insects (mosquitoes) |
| *Phasivirus* | PCLPV | *Phasi Charoen-like phasivirus* | AKP18602.1 | Insects (mosquitoes) |
| *Phasivirus* | WFV | *Wuhan fly phasivirus* | AJG39259.1 | Insects (mosquitoes) |
| *Tenuivirus* | RaSV | *Ramu stunt virus* | ALJ83282.1 | Arthropod vectors (Hemiptera), plant hosts |
| *Tenuivirus* | RGSV | *Rice grassy stunt tenuivirus* | BAA89602.1 | Arthropod vectors (Hemiptera), plant hosts |
| *Tenuivirus* | RiSV | *Rice stripe tenuivirus* | AFM93792.1 | Arthropod vectors (Hemiptera), plant hosts |
| *Goukovirus* | Cumuto | *Cumuto virus* | AHH60917.1 | Insects (mosquitoes) |
| *Goukovirus* | Gouleako | *Gouleako virus* | AEJ38175.1 | Insects (mosquitoes) |
| *Goukovirus* | YIV | *Yichang insect virus* | AJG39273.1 | Insects (mosquitoes) |
| *Leishbunyaviridae* | ABV1 | *Apis bunyavirus 1* | ARO50045.1 | Honey bee (*Apis mellifera*) + Trypanosomatidae (*Lotmaria passim*) |
| *Leishbunyaviridae* | CG15LBV1 | *Crithidia sp. G15 Leishbunyavirus 1* | | Trypanosomatidae |
| *Leishbunyaviridae* | LmorLBV1b | *Leptomonas moramango Leishbunyavirus 1b* | | Trypanosomatidae |
| *Leishbunyaviridae* | CC4LBV1 | *Crithidia sp. C4 Leishbunyavirus 1* | | Trypanosomatidae |
| *Leishbunyaviridae* | CZMLBV1 | *Crithidia sp. ZM Leishbunyavirus 1* | | Trypanosomatidae |
| *Leishbunyaviridae* | CabsLBV1 | *Crithidia abscondita Leishbunyavirus 1* | | Trypanosomatidae |
| *Leishbunyaviridae* | LmorLBV1a | *Leptomonas moramango Leishbunyavirus 1a* | | Trypanosomatidae |
| *Leishbunyaviridae* | DuBV | *Duke bunyavirus* | ARE30258.1 | Honey bee (*Apis mellifera*) + ? |
| *Leishbunyaviridae* | CpraLBV1 | *Crithidia pragensis Leishbunyavirus 1* | | Trypanosomatidae |

**Table S2. Cont.**

| Clade | Short name | Virus name | Accession no. | Host |
|---|---|---|---|---|
| *Leishbunyaviridae* | HBLV5 | *Hubei bunya-like virus 5* | APG79301.1 | Insects (Diptera) + Trypanosomatidae (Strigomonadinae) |
| *Leishbunyaviridae* | CotoLBV1 | *Crithidia otongatchiensis Leishbunyavirus 1* | | Trypanosomatidae |
| *Leishbunyaviridae* | HBLV6 | *Hubei bunya-like virus 6* | APG79326.1 | Horse leech (*Whitmania pigra*) + Trypanosomatidae (*Trypanosoma* sp) |
| *Leishbunyaviridae* | PTCCLBV1 | *Phytomonas sp. TCC231 Leishbunyavirus 1* | | Trypanosomatidae |
| *Leishbunyaviridae* | HHFV | *Huangshi Humpbacked Fly virus* | AJG39239.1 | Humpbacked Fly (*Megaselia scalaris*) + Trypanosomatidae (Strigomonadinae) |
| *Leishbunyaviridae* | WSV | *Wuhan Spider virus* | AJG39269.1 | Arthropods (Araneae) + *Diptea*, *Heteroptera* + Trypanosomatidae (*Herpetomonas* sp) |
| *Nairoviridae*, *Orthonairovirus* | DONV | *Dugbe orthonairovirus* | AMT75392.1 | Arthropod vectors (ticks), vertebrate hosts |
| *Nairoviridae*, *Orthonairovirus* | CCHFONV | *Crimean-Congo hemorrhagic fever orthonairovirus* | ARB51463.1 | Arthropod vectors (ticks), vertebrate hosts |
| *Arenaviridae* | LCMAV | *Lymphocytic choriomeningitis mammarenavirus* | AMR60827.1 | Vertebrates |
| *Arenaviridae* | MaMAV | *Machupo mammarenavirus* | AMZ00419.1 | Vertebrates |
| *Arenaviridae* | GGV | *Alethinophid 1 reptarenavirus (Golden Gate virus)* | AFP93553.1 | Vertebrates |
| *Arenaviridae* | Boa | *Alethinophid 3 reptarenavirus (Boa arenavirus)* | AGH06042.1 | Vertebrates |
| *Phasmaviridae*, *Orthophasmavirus* | KPOFV | *Kigluaik phantom orthophasmavirus* | AIA24559.1 | Insects |
| *Phasmaviridae*, *Orthophasmavirus* | WCOPV | *Wuchang cockroach orthophasmavirus 1* | AJG39258.1 | Insects |
| | FERV | *Ferak orthoferavirus* | AKN56913.1 | Insects |
| | JONV | *Jonchet orthojonvirus* | AKN56884.1 | Insects |
| *Hantaviridae*, *Orthohantavirus* | HOHV | *Hantaan orthohantavirus* | APH07644.1 | Vertebrates |
| *Hantaviridae*, *Orthohantavirus* | KhaOHV | *Khabarovsk orthohantavirus* | AIL25337.1 | Vertebrates |
| *Fimoviridae*, *Emaravirus* | EMARAV | *European mountain ash ringspot-associated virus* | AAS73287.2 | Arthropod vectors (Eriophyidae), Plant hosts |
| *Fimoviridae*, *Emaravirus* | RREV | *Rose rosette emaravirus* | ADZ54688.1 | Arthropod vectors (Eriophyidae), Plant hosts |
| *Tospoviridae*, *Orthotospovirus* | TSWV | *Tomato spotted wilt orthotospovirus* | AIY28466.1 | Arthropod vectors (Thripidae), Plant hosts |
| *Tospoviridae*, *Orthotospovirus* | IYSV | *Iris yellow spot orthotospovirus* | ACM89280.1 | Arthropod vectors (Thripidae), Plant hosts |
| *Peribunyaviridae* | HeHV | *Herbert herbevirus* | AGX32061.1 | Insects |
| *Peribunyaviridae* | TaHV | *Tai herbevirus* | AGX32057.1 | Insects |
| *Peribunyaviridae* | BUNV | *Bunyamwera virus* | AKX73309.1 | Arthropod vectors (ticks), vertebrate hosts |
| *Peribunyaviridae* | OROV | *Oropouche orthobunyavirus* | ALB07205.1 | Arthropod vectors (ticks), vertebrate hosts |
| Narnaviruses of trypanosomatids (Fig. 4*D*) | | | | |
| *Ourmia-like viruses* | SAOV1 | *Soybean-associated ourmiavirus 1* | ALM62238 | Plants |
| *Ourmia-like viruses* | SAOV2 | *Soybean-associated ourmiavirus 2* | ALM62250 | Plants |
| *Ourmia-like viruses* | SsOLV1 | *Sclerotinia sclerotiorum ourmia-like virus 1* | ALD89138 | Fungi |
| *Ourmia-like viruses* | RsOLV1 | *Rhizoctonia solani ourmia-like virus 1* | ALD89131 | Fungi |
| *Ourmia-like viruses* | BOV | *Botrytis ourmiavirus* | CEZ26310 | Fungi |
| *Ourmia-like viruses* | SsOLV2 | *Sclerotinia sclerotiorum ourmia-like virus 2* | ALD89139 | Fungi |
| *Ourmiavirus* | CsVC | *Cassava virus C* | ACI03053 | Plants |
| *Ourmiavirus* | EpCV | *Epirus cherry virus* | ACF16357 | Plants |
| *Ourmiavirus* | OuMV | *Ourmia melon virus* | ACF16360 | Plants |
| *Narnavirus* | ScNV-20S | *Saccharomyces cerevisiae 20S RNA narnavirus* | AAC98925 | Fungi |
| *Narnavirus* | ScNV-23S | *Saccharomyces cerevisiae 23S RNA narnavirus* | AAC98708 | Fungi |
| *Narnavirus* | NarEnv | *Narnaviridae environmental sample* | AJT39596 | Unknown |
| *Narnavirus* | PserNV1 | *Phytomoas serpens narnavirus 1* | | Trypanosomatidae |
| *Narnavirus* | LepseyNLV1 | *Leptomonas seymouri narna-like virus 1* | | Trypanosomatidae |

**Table S2. Cont.**

| Clade | Short name | Virus name | Accession no. | Host |
|---|---|---|---|---|
| *Narnavirus* | PiRV4 | *Phytophthora infestans RNA virus 4* | AEM89291 | Oomycetes |
| *Narnavirus* | TSA: Tdalm | *Teleopsis dalmanni transcribed RNA* | GBBP01132666.1 | *Teleopsis dalmanni* + Trypanosomatidae (*Jaenimonas* sp.) |
| *Mitovirus* | CcMV1a | *Cryphonectria cubensis mitovirus 1a* | AAR01970 | Fungi (mitochondria) |
| *Mitovirus* | SsMV9 | *Sclerotinia sclerotiorum mitovirus 9* | AHF48625 | Fungi (mitochondria) |
| *Mitovirus* | BcMV3 | *Botrytis cinerea mitovirus 3* | CEZ26302 | Fungi (mitochondria) |
| *Mitovirus* | OMV3a | *Ophiostoma mitovirus 3a* | CAA06228 | Fungi (mitochondria) |
| *Mitovirus* | SsMV3 | *Sclerotinia sclerotiorum mitovirus 3* | AGC24232 | Fungi (mitochondria) |
| *Levivirus* | GA | *Enterobacteria phage GA* | CAA27499 | Bacteria |
| *Levivirus* | MS2 | *Enterobacteria phage MS2* | P00585 | Bacteria |

CBPV, *Chronic bee paralysis virus*.

**Table S3. Accession numbers and/or sequences reported in this work**

| Species | Isolate | Viral name | Accession nos. |
|---|---|---|---|
| *Crithidia otongatchiensis* | Ecu-08 | *Coto*LBV1 | KX373292, KX451144, KX451145, KX683300 |
| *Crithidia* sp. | G15 | CG15LBV1 | KX373291 |
| *Crithidia* sp. | ZM | CZMLBV1 | KX373293 |
| *Crithidia* sp. | C4 | CC4LBV1 | KY322668 |
| *Crithidia abscondita* | 127AL | CabsLBV1 | KX507299, KX507300, KX507301 |
| *Crithidia pragensis* | MCZ-11 | CpraLBV1 | KY322669 |
| *Leptomonas moramango* | MMO-09 | LepmorLBV1 | KX280012-KX280017 |
| *Phytomonas* sp. | TCC231 | PTCCLBV1 | KY322667 |
| *Leptomonas pyrrhocoris* | F165 | LeppyrTLV1 | KX373302, KX373303 |
| *Leptomonas pyrrhocoris* | F19 | LeppyrTLV1 | MG008317 |
| *Leptomonas pyrrhocoris* | H10 | LeppyrTLV1 | KX373300, KX373301 |
| *Phytomonas serpens* | 9T (UCR) | PserNV1 | KU882057, KY322666 |
| *Phytomonas serpens* | 30T | PserNV1 | — |
| *Leptomonas seymouri* | ATCC | LseyNLV1 | KU935604, KU935605, KX373304, KX373305 |
| *Leptomonas pyrrhocoris* | F19 | LeppyrOV1 | — |
| *Leptomonas pyrrhocoris* | H10 | LeppyrOV1 | KX373294-KX373299 |
| *Leptomonas pyrrhocoris* | P59 | LeppyrTLV1-EVE1 | KY364836, KY364842 |
| *Leptomonas pyrrhocoris* | PP1 | LeppyrTLV1-EVE1 | KY364837, KY364843 |
| *Leptomonas pyrrhocoris* | PP2 | LeppyrTLV1-EVE1 | KY364835, KY364841 |
| *Leptomonas pyrrhocoris* | LP | LeppyrTLV1-EVE1 | KY364839 |

**Table S4. Metatranscriptomic contigs assembled from SRA depositions**

| | | | | Source of the contig | | | |
|---|---|---|---|---|---|---|---|
| Name of contig | Sequence | Identity | SRA accession | Name of virus found in the SRA | SRA name | Biomaterial included in the sample | Ref. to the SRA |
| SRX1712638_HBLV6_PFR | GATTGCGAAGCTGGAGAAGATTGAGGATGAGCTGCGCCGCTCTCAGCTAGACGCG-ACGGAAATGGCACAGACGCCAGTGATTGTGCTCAAGAACCTCGAG | *Trypanosome* paraflagellar rod protein 2 | SRX1712638 | *Hubei bunya-like virus 6* (HBLV6) | Leech mix Hubei | *Whitmania pigra* (horse leech) | Shi (2016) (1) |
| SRX833697_WSV_18S | CCATGCGCAGATCAGACGTAATCTGCCGCAAAAATTTTGCGGTTTCCGCAACATT-GGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAACCAGGCGTTCTCCG-CCACGGGCGTGCGGGCAACCGTACGTCTAGTGAGACGCCTTGCGAATGAATGA-CATTAAAACCAATGCCTTCACTGGCAGTAACACCCAGAAGTGTTGACTCAATT-CATTCCGTGCGAAAGCCGGATTTCCGGCGTCTTTTGACGAACAACTGCCCTAT-CAGCTAGTGATGGCAGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGA-TTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGA-GGGC | *Herpetomonas* sp. 18S ribosomal RNA | SRX833697 | *Wuhan spader virus* (WSV) | Spiders | *Neoscona*, *Parasteatoda*, *Plexippus*, *Pirata*, Araneae spp. | Li (2015) (2) |
| SRX1711976_HBLV5_18S | ATCAGCTCGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGA-TTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGA-GGGCAGCAGGCGCGCAAATTGCCCAATGTCAAGAAAAAACGATGAGGCAGCGA-AAAGAAAT | Strigomonadinae 18S ribosomal RNA | SRX1711976 | *Hubei bunya-like virus 5* (HBLV5) | Diptera mix Hubei | *Drosophila*, *Episyrphus*, *Sarcophaga*, *Muscina*, *Ptecticus* | Shi (2016) (1) |
| SRX833692_HHFV_18S | CCGGCGTCTTTTGACGAACAACTGCCCTATCAGCTAGTGATGGCCGTGTAGTGGA-CTGCCATGGCGTTGACGGGAGCGGGGGATTAGGGTTCGATTCCGGAGAGGGAG-CCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAGGCGCGCAGGCGCGCAAATTGCCCA-ATGTCAAGAAAAAACGATGAGGCAGCGAAAAGAAATAGGTTTG | Strigomonadinae 18S ribosomal RNA | SRX833692 | *Huangshi humpbacked fly virus* (HHFV) | Insects mix 4 (insect in the mountain) | *Psychoda*, *Velarifictorus*, *Crocothemis*, Phoridae spp., Lampyridae spp., *Aphelinus*, *Hyalopterus*, *Aulacorthum* | Li (2015) (2) |
| SRX2422212_ABV1_colonyA_18S | TGACAGTAAAACCAATGCCTTCACTGGCAGTAACACCCAGACGTGTTGACTCAAT-TCATTCCGTGCGAAAGCCGGCTTGTTCCGGCGTCTTTTGACGAACAACTGCCC-TATCAGCTGGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGG-GGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTAC-GGAGGGCAGCAGGCGCGCAAATTGCCCAATGTCAAAACAAAACGATGAGGCAG-CGAAAAGAAATAGAGTTGTCAGTCCATTTGGATTGTCATTTCAATGAGGGATA-TTTAAACCCATCGAAAATCTAGTAACAATTGGAGGACAAGTCTGGTGCCAGCA-CCCGCGGTAATTCCAGCTCCAAAAGCGTATAT | Leishmaniinae 18S ribosomal RNA | SRX2422225 | *Apis bunyavirus 1* | RNA-seq of Apis mellifera: South Africa colony 11 | *Apis mellifera* | Remnant (2017) (3) |
| SRX833692_HHFV_nuc | GGAGGACGATCTGCAGATTCAATATGTCTGCCTTAGAGGTTGAAGAGATTTGTTT-TAACCTAGCCTATGTGGCTGTGACGCCTTGGGACACACGTGTGGCCTTAGAGA-GAGATGGAAGGTCCGAGGTTGCGAAGTTAATAGCCATCGTTGTGGGCTGCAGG-GGTACCAACCTCGCCAAGGTCGTTAAAAGGAGTAATAATCCTGAAAAGGCCCT-CGAATACGTGAATAGCATCCGCTCTTTCATGGCCAACCAGACTGTCTCCTTGG-GNNCTGTCTCCTTGGGACATATTGCCGCCGCTTACCCCGAGGTAGTCTACGAT-GCAAGAATTGCGTCCAAGAGTGCTATCTCTGTCAACACCCTCCAGTTCCTGAA-ATTCGCAGGTCTGACCAAGACGGTGTGGCTGAAGGCTAATCACGACTTCTTCG-AACTCACAGGCCAAAGTTCAGAGAAGTTTGATGAGCTGTCCGATGTGATATGG-GCAGACGATAGCTTCGCTGGCGTGGTAGGTCACAGG | LBV nucleocapsid | SRX833692 | *Huangshi humpbacked fly virus* (HHFV) | Insects mix 4 (insect in the mountain) | *Psychoda*, *Velarifictorus*, *Crocothemis*, Phoridae spp., *Lampyridae* spp., *Aphelinus*, *Hyalopterus*, *Aulacorthum* | Li (2015) (2) |
| SRX833697_WSV_nuc | GGGGGATTGGAGAATCAGGCAAAACTGGTTGCGATGATCGTCGGGTGCAGGGGAA-CCAATCTTGATAAGATCCAAGGTTCTTCGAGCAATCCGGAGATGGCACGTCGC-CTTGTTGGCATTGCCCGTAGCCTGTCAGCAGACCTGGGCGTCTCAGTGGCTCA-CATTGCTTCTGCTTATCCAGAGGTGCTTTATGATGCCAGGAAGAGGTGCGGCA-AGTCAGATAAGTGTGAACACTATTTCTTCCTTGAAGGTGACGGCCTCACCAAG-GATGCTTGGCTGAGGGCAAACAGGGAGTTCTGCCAGCTGGTGGGGCTTGACTA-CCAGAAGTTTCAACGCATTTCTGAAATGATCTGGTCTGATAGCAGCTCATCGCCC | LBV nucleocapsid | SRX833697 | *Wuhan spader virus* (WSV) | Spiders | *Neoscona*, *Parasteatoda*, *Plexippus*, *Pirata*, Araneae spp. | Li (2015) (2) |
| SRX1711976_HBLV5_nuc_contig6 | ATGTCTGAGCCAGCTGGAGATAGCCACATGATGCTTGAGCTTGACGATATAGTTG-AAAGCCTTAAGTATCAGGGGTTGAGTCCATGGGAAACGAGAGAGAGGATGTGC-AGAGAAGGTCATGAGAAGATGGGGAGGCTTATTGGCTATGTAGTAGGATTGAG-GGGAACGAACCTTGCTAAAATGGCTTATAAGAGTGTGGATCGGGAGAAGGCTG-CAGTGATCGTGGAGAGAATCAATAGGTATATGGTAACTCAGCCTGTATCTATT-GGACATATCGCTGCAGCTTTTCCTGATTGTGTTTACGATGCAAGACTTAGAAC-TGAAAACAGAAGATTTGTGAATACGCTACAGTTCTTAAAGCACAATGGTCTGC-GGAAAGAGGTTTGGTTTCAAGCTAATTTAGATTTCTTACAAACCACGAGGCAA-GATGCTAGCAAGTTTGTGGATCTGGCAGATATTATTTGGAGAGATGATAGTTT-TGCTGGGATTGTGGGAGATAGGATGCCTTGTTAGTCAGTTGCTTAGAAACCTA-TGGAATTCAAGTTATGGCGC | LBV nucleocapsid | SRX1711973 | *Hubei bunya-like virus 5* (HBLV5) | Diptera mix Hubei | *Drosophila*, *Episyrphus*, *Sarcophaga*, *Muscina*, *Ptecticus* | Shi (2016) (1) |

**Table S4. Cont.**

| Name of contig | Sequence | Identity | SRA accession | Source of the contig: Name of virus found in the SRA | Source of the contig: SRA name | Source of the contig: Biomaterial included in the sample | Source of the contig: Ref. to the SRA |
|---|---|---|---|---|---|---|---|
| SRX1711976_HBLV5_nuc_contig15 | CAATTTTTGATTTAAACGGCAGGTTGGCACTATCCCTCACCGGCCAAATATTAAG-<br>CTGGACCAGCTGGGAGCCTATCACCAACAATTTTGGAGAAAGAATCATCATCC-<br>CAAATTGCATCTGAAACGGCAAGAAACCGGTCCGGACTTAGATGCACTAAACG-<br>ACAAAAATCCAAGTTCGCCTGTAGCCACACGTCTTTGGTAAGTCCATTAAATT-<br>TCAAGAACTGAAGTGTGTTCACTGAGATTAAAGAACTGCATTCAACCCGCACA-<br>TCGTACATCACCTCGGGGAAAGCTGAAGCAATGTGTGCTGCAGAGACTCCATA-<br>AAGAGCCACCATTTCTCTTACAACAGCAATGAGCTCTGCAGCCTTCTGTTTGT-<br>CCTTCGAGCGACCAGCAATCTTATCTAGCCTTGTTCCTCGAGCTGCGACCAAG-<br>ATAGCAACAATTTTGGCTTTCTCCAAATGTCCTGCTTGGATAATCTTCATCCT-<br>AGTGGACCATGGTGATACCCCCTCGTATTGGATTGCATCAAGTAGCGCAATCT-<br>CTTTCGTGTCTAGGGAGGAGCTCATTTTTCTCTCTATAGGGAAAATAGTGCTT-<br>TCGTCTGG | LBV nucleocapsid | SRX1711974 | *Hubei bunya-like virus 5* (HBLV5) | Diptera mix Hubei | *Drosophila*, *Episyrphus*, *Sarcophaga*, *Muscina*, *Ptecticus* | Shi (2016) (1) |
| SRX1711976_HBLV5_nuc_contig18 | GCCTTTCTTACTCTTCCTTCGTCGATGCAGTCGGTGGCCTCGAGGCTCTGGCTGA-<br>AGCTCTCAGCTATAGGGGTAGAAGTCCCTACCAGACTCGAGAGCTGCTGATAT-<br>CCGAAGGACTAGAAGGACCGGCCAGAATTCTTTCTTTCACCGTAGGTTGTCGG-<br>GGCACCAATCTTATGAAGCTCCGAGAAAAGAGTGAGAAGTCAGAAGTACTCGA-<br>TAGAGTGCTTGCTGCTTTGTCCTTGGTGACCAGTGCTCACAAAGAGTCCTTAG-<br>GTCATCTGGCTGCTGCTTTCCCTGAGAACTTGTATGATGGTAGAGTGGTGAGC-<br>AATTCGCCCTTCTCCGTCAACACTATCCAGGCTCTGAAGTTCAATGGCTTGGA-<br>GAAGAGTGACTGGGTAGCAGCCAACATCAAAATGCTGGAGTCAGTGAAGGGTG-<br>ATGTTGCGAAGTTCACAGCCATCGCTGATATTGTTTGGGGAGACGACAAGTTT-<br>TCAGGAGTGATGGGAGATAGGAAGCCCTCCCCTGTGTAATTAGCGACTGAATC-<br>TACCAATTAGAGGGAGTGTTAGTTGTAGATGTAGT | LBV nucleocapsid | SRX1711975 | *Hubei bunya-like virus 5* (HBLV5) | Diptera mix Hubei | *Drosophila*, *Episyrphus*, *Sarcophaga*, *Muscina*, *Ptecticus* | Shi (2016) (1) |
| SRX1711976_HBLV5_nuc_contig8 | CTCCAACCACTCCTGCGAAGCGGTCGTCCTCCCAGATCAAGTCCGCAATGGACTC-<br>AAATCTGGGGATCTCCCCCGCCCTCTTCACGAGGGTCAACATCTCACGATTGG-<br>CTTTGAGCCAATCATCCTTCTCAAGCCCAGCGTGCTTGAGGAAGGCGAGCGTG-<br>TTGACACTAACGGGGGAGCCGCCCGCAACTCTCCCATCATAGACCTCCTCCGG-<br>GAAGGCCGCCGCGAGGTGCCCTAAAGACACCCCGTACGTCGCCACAAGAGCCC-<br>TGGCATTAGCTGCCAGGGCCCGGGCGACCTCAGGACCTTTACTCCGG | LBV nucleocapsid | SRX1711976 | *Hubei bunya-like virus* 5 (HBLV5) | Diptera mix Hubei | *Drosophila*, *Episyrphus*, *Sarcophaga*, *Muscina*, *Ptecticus* | Shi (2016) (1) |
| SRX1712638_HBLV6_nuc | CCCCCACCTAGGATGAATTTGAGCACTATTTCCTCCGCTGACGTTACAGCATTCT-<br>CAAATGAAGAGATGACTATTGCCCTAAATCAAATCACTACAGATATCCATTAT-<br>GAAGGTAAGTCTCCTGAGCAAACTAGGATAGACATAGAGAATGCTCACTTAGG-<br>TAATGCTGCTAGAATCATAGCTTACACTGTGGGGACTGCTGGAACCAATTGGG-<br>AAAGCAATGCACATAAGTTCTCTAATAGTCAAACATTAGTGAACTTCATACGA-<br>CTTCATACTGCAATATGTACTAGTTTGCGTAGACAAGTCTCATTGGCACACAT-<br>AGCAGCAGCATACCCAGCAGAGTTGTATGATGGTAGAAAGGCATCTCGCAGTC-<br>AAATCTCTGTTGATACGATCCCTAGTCTGAAGCACAAAGGGCTAACAAAAGAA-<br>CGGTGGCTAGAAGCAACTGAGAATTTTATCCGATTAACTTCAAGCACCCCTGC-<br>CACAGCATTATTCAATTTTTACAAAATAGCTGATATGATCTGGAATAATGCAC-<br>CTGACATAATGGGTGATAGGGATCCTGAAAATCCA | LBV nucleocapsid | SRX1712638 | *Hubei bunya-like virus 6* (HBLV6) | Leech mix Hubei | *Whitmania pigra* (horse leech) | Shi (2016) (1) |

Partial sequences of trypanosomatid-specific and LBV-like nucleocapsid protein genes assembled from viral metatranscriptomes. Because they have not been experimentally verified, they cannot be deposited in GenBank.

1. Shi M, et al. (2016) Redefining the invertebrate RNA virosphere. *Nature* 540:539–543.
2. Li CX, et al. (2015) Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *Elife* 4:e05378.
3. Remnant EJ, et al. (2017) A diverse range of novel RNA viruses in geographically distinct honey bee populations. *J Virol* 91:e00158-17.

**Table S5. Codon usage in LeppyrOV1 and LeppyrTLV1 ORFs**

| Sequence | T3 | C3 | A3 | G3 | GC |
|---|---|---|---|---|---|
| LeppyrOV1 | | | | | |
| ORF1 | 0.40 | 0.30 | 0.18 | 0.31 | 0.51 |
| ORF2 | 0.35 | 0.32 | 0.19 | 0.33 | 0.53 |
| ORF3 | 0.39 | 0.28 | 0.20 | 0.34 | 0.52 |
| ORF4 | 0.33 | 0.34 | 0.17 | 0.34 | 0.55 |
| ORF5 | 0.39 | 0.27 | 0.15 | 0.33 | 0.57 |
| ORF6 | 0.45 | 0.27 | 0.16 | 0.32 | 0.48 |
| Average | 0.38 | 0.30 | 0.18 | 0.33 | 0.53 |
| LeppyrTLV1 | | | | | |
| ORF1 | 0.34 | 0.26 | 0.33 | 0.32 | 0.45 |
| ORF2 | 0.41 | 0.29 | 0.28 | 0.23 | 0.47 |
| ORF3 | 0.34 | 0.24 | 0.20 | 0.22 | 0.47 |
| Average | 0.36 | 0.26 | 0.27 | 0.26 | 0.46 |
| Leppyr genome | | | | | |
| | 0.14 | 0.38 | 0.1 | 0.39 | 0.77 |

A3, C3, G3, and T3 are frequencies of the corresponding nucleotides in the third position; GC is the overall GC content.

**Table S6. Complementary terminal sequences (panhandles) of LBV1s and other Bunyavirales**

| Virus | 5′ | 3′ |
|---|---|---|
| LBV1s | | |
| L segment | | |
| CG15LBV1 | ACACAAAGAGAA | TGTTCTTTGTGT |
| LepmorLBV1b | ACACAAAGACAA | TATTCTTTGTGT |
| LepmorLBV1a | ACACAAAGATAA | ND |
| CZMLBV1 | ACACAAAGAGAA | TGTTCTTTGTGT |
| CotoLBV1 | ACACAAAGACGA | TATTCTTTGTGT |
| PTCCLBV1 | ACACAAAGAAGA | TATTCTTTGTGT |
| Consensus | ACACAAAGANAA | TRTTCTTTGTGT |
| M segment | | |
| CabsLBV1-M | ACACAAAGAGAA | TGTTCTTTGTGT |
| CotoLBV1-M | ACTCAAAGACGA | ND |
| LepmorLBV1a-M | ACACAAAGATAA | TATTCTTTGTG- |
| LepmorLBV1b-M | ACACAAAGACAA | ND |
| consensus | ACWCAAAGABRA | TRTTCTTTGTGT |
| S segment | | |
| CabsLBV1-S | ACACACGGAAAA | TATTCTTTGTGT |
| CotoLBV1-S | CACAACGACGA | ND |
| LepmorLBV1a-S | ACACATAGACAA | ND |
| LepmorLBV1b-S | ACACACAGATAA | ND |
| Consensus | ACACAHVGAHRA | TRTTCTTTGTGT |
| *Phenuiviridae* | | |
| *Gouleako virus* | | |
| L segment | ACACAAAGACAC | TGGACTTTGTGT |
| M segment | ACACAGTGACCC | GGGACTTTGTGT |
| S segment | ACACAGTGACCT | GGGACTTTGTGT |
| *Rift Valley fever virus* | | |
| L segment | ACACAAAGGCGC | CGGTCTTTGTGT |
| M segment | ACACAAAGACGG | CGGTCTTTGTGT |
| S segment | ACACAAAGACCC | GGAGCTTTGTGT |
| *Rice grassy stunt virus* | | |
| L segment | ACACAAAGTCCT | CAGACTTTGTGT |
| M segment | ACACAAAGTCCT | CAGACTTTGTGT |
| S segment | ACACAAAGTCCT | CAGACTTTGTGT |
| Other Bunyavirales | | |
| *Hantaan orthohantavirus* | | |
| L segment | TAGTAGTAGACT | AGCATACTACTA |
| M segment | TAGTAGTAGACT | AGCATACTACTA |
| S segment | TAGTAGTAGACT | AGCATACTACTA |
| *Crimean-Congo hemorrhagic fever orthonairovirus* | | |
| L segment | TCTCAAAGATAT | ATTTCTTTGAGA |
| M segment | TCTCAAAGAAAT | ATTTCTTTGAGA |
| S segment | TCTCAAAGAAAC | ATTTCTTTGAGA |

Alignments of 5′ and 3′ terminal sequences L, M, and S segments of LBV1s and selected other Bunyavirales are shown. Nucleotides involved in bulge formation are underlined. ND, not determined.