Detection of a novel parahenipavirus from northern short-tailed shrews (*Blarina brevicauda* [Say, 1823])

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Abstract

Parahenipaviruses (genus *Parahenipavirus*) are non-segmented negative strand RNA viruses belonging to the family *Paramyxoviridae* of the order *Mononegavirales*. Parahenipaviruses have been detected in shrews and rodents, some of which have been reported to be associated with human diseases. Although many parahenipaviruses have been identified thus far, there still exist large phylogenetic gaps in parahenipaviruses, suggesting the existence of yet-to-be-identified parahenipaviruses. In this study, we analyzed public RNA-seq data and identified a novel parahenipavirus from northern short-tailed shrews (*Blarina brevicauda* [Say, 1823]), tentatively named Blarina brevicauda virus (BbV). Based on sequence comparisons between BbV and its most closely related viruses, BbV meets the International Committee on Taxonomy of Viruses species demarcation criteria, and therefore we propose that BbV is a novel species of virus in the genus *Parahenipavirus*. Furthermore, mapping analysis using RNA-seq data derived from multiple tissues of northern short-tailed shrews suggested that BbV is kidney-tropic. This study provides novel insights into the diversity of parahenipaviruses, which would contribute to a deeper understanding of their evolution and control of infectious diseases.

36 **Introduction**

37 The genus *Parahenipavirus* is a newly established genus within the family *Paramyxoviridae* of
38 the order *Mononegavirales*, consisting of viruses relatively closely related to the members of the
39 genus *Henipavirus* [1]. The genome of parahenipaviruses contains conserved genes typical of
40 paramyxoviruses, including N, P/V/W/C, M, F, G/H/HN, and L, arranged from the 3' end.
41 Additionally, an additional ORF, X, located between the M and F genes, encodes a putative
42 transmembrane protein that is unique to parahenipaviruses within the family *Paramyxoviridae*
43 [2]. The C, V, and W genes overlap with the P gene; the C protein is derived from an alternative
44 reading frame of P, while the V and W proteins are expressed through mRNA editing, involving
45 the insertion of one or two G residues, respectively.

46 Parahenipaviruses have been mainly detected in shrews and rodents [2–6], some of which
47 are associated with human diseases. In 2022, Langya virus was detected in febrile patients in
48 China, with shrews identified as the natural reservoir [3]. Similarly, Mojiang virus was detected
49 in rats from an abandoned mine where three patients died of severe pneumonia, although a
50 direct causal link remains unclear [4]. While other parahenipaviruses have not been linked to
51 human disease, their natural hosts, such as rodents and shrews, frequently come into contact
52 with humans, highlighting the need to elucidate the diversity of parahenipaviruses for
53 controlling viral infectious diseases.

54 The diversity of parahenipaviruses is not fully understood. To date, 12 species and several
55 unclassified viruses have been identified [1]. However, significant phylogenetic gaps within the
56 genus *Parahenipavirus* suggest the existence of many unidentified viruses. Further exploration
57 is needed to deepen our understanding of parahenipavirus diversity and evolution as well as to
58 control infectious diseases.

59 Sequencing data from diverse samples have been deposited in public databases, some of
60 which may contain virus-derived sequences. Efforts to identify viruses using publicly available
61 data have led to the discovery of many novel viruses, demonstrating the usefulness of re-

62 analyzing archived data for virus discovery [7]. As new public data continue to accumulate over
63 time, they could serve as a valuable resource for identifying additional novel viruses.
64 In this study, we searched for parahenipaviruses using public RNA-seq data and identified
65 a novel parahenipavirus from the northern short-tailed shrew (*Blarina brevicauda* [Say, 1823]).
66 We determined the nearly complete genome sequence of the virus and tentatively named it
67 Blarina brevicauda virus (BbV). Mapping analyses revealed that viral reads were predominantly
68 detected in kidney-derived data, suggesting that BbV is kidney-tropic. Furthermore, this virus
69 meets the International Committee on Taxonomy of Viruses (ICTV) species demarcation
70 criteria, and we propose BbV as a novel species of virus within the genus *Parahenipavirus*.
71

72 **Materials and Methods**

73 ***Identification of Blarina brevicauda virus from public RNA-seq data***

74 RNA-seq data (SRR17216496) was downloaded from NCBI SRA [8] and preprocessed using
75 fastp (v0.23.4) [9] with the options "-l 35 -x -y". The preprocessed reads were then assembled
76 using SPAdes (v4.0.0) [10] with default settings. Contigs of 100 nucleotides or longer were
77 extracted using SeqKit (v2.4.0) [11] and subjected to a BLASTx [12] search against the NCBI
78 clustered nr database [8] on July 29, 2024.
79 To validate the viral contig, we mapped the RNA-seq data back to the contig using Magic-
80 BLAST (v1.7.2) [13] with the options "-no_unaligned -splice T -word_size 16 -lcase_masking -
81 limit_lookup F". Read depth at each contig position was calculated using SAMtools (v1.19)
82 [14]. Positions with a read depth of 5 or greater were considered reliable, and regions with a
83 read depth below 5 were removed. The mapping pattern was visualized and manually inspected
84 using Geneious Prime (v2023.2.1, https://www.geneious.com). The final contig, deposited in
85 DDBJ under the accession number BR002473, was used for downstream analyses.
86

87 ***Annotation of Blarina brevicauda virus genome***

88 For viral gene annotation, ORFs longer than 300 nucleotides were extracted. The putative amino
89 acid sequences of these ORFs were subjected to BLASTp searches against viral sequences
90 (taxid: 10239) in the NCBI nr database, using the options "-word size 2 -evalue 1E-10." Viral
91 genes were annotated based on the BLAST results.
92 To identify putative transcription signal sequences, the intergenic regions were extracted
93 and aligned using MAFFT (v7.490) [15] with the E-INS-i algorithm. Based on this alignment
94 and known paramyxovirus signal sequences, the putative transcription signal sequences were
95 manually determined.
96 The transmembrane domains were predicted using the DeepTMHMM webserver (v1.0.42)
97 [16].
98
99 ***Phylogenetic analyses***
100 Amino acid sequences of the N and L proteins from BbV, other parahenipaviruses,
101 henipaviruses, and three viruses from other genera within the family *Paramyxoviridae*
102 (Supplementary Table 1) were aligned using MAFFT (v7.490) with the E-INS-i algorithm and
103 ambiguously aligned regions were trimmed with trimAl (v1.4.rev22) [17] using the "-strict"
104 option. Phylogenetic trees were inferred by the maximum likelihood method using RAxML-NG
105 (v1.1.0) [18]. The LG+I+G4+F model, selected by ModelTest-NG (v0.2.0) [19], was used for
106 tree reconstruction. The reliability of the phylogenetic tree was assessed by 1,000 bootstrap
107 resamplings using the transfer bootstrap expectation method. The alignments used for the
108 phylogenetic analyses are available in the Supplementary materials.
109
110 ***Calculation of amino acid sequence identities for classification***
111 Following ICTV species demarcation criteria
112 (https://ictv.global/ictv/proposals/2023.018M.Paramyxoviridae_reorg.zip [accessed on Sep 22,
113 2025]), we performed BLASTp searches against viral proteins (taxid: 10239) in the NCBI

114 nonredundant (nr) database using the BbV L-protein sequence as the query, identifying
115 Henipavirus sp. LS-SCDW-1 (PQ421759.1) as a top hit. However, phylogenetic analysis based
116 on L-protein sequences indicated that Ninorex virus (OQ438286.1) is more closely related to
117 BbV. Accordingly, both viral sequences were included in subsequent analyses. Pairwise
118 BLASTp alignments were performed between BbV and Henipavirus sp. LS-SCDW-1 or
119 Ninorex virus for the six major proteins of paramyxoviruses (N, P, M, F, G, and L), and the
120 average amino acid sequence identity was calculated.
121
122 ***Mapping analysis for detection of Blarina brevicauda virus-derived reads***
123 To detect Blarina brevicauda virus-derived reads, we mapped 306 publicly available RNA-seq
124 datasets obtained from animals in the order *Eulipotyphla* (Supplementary Table 2) to the BbV
125 genome sequence using Magic-BLAST (v1.7.2). The number of mapped reads was then counted
126 using SAMtools (v1.19).
127
128 **Results**
129 ***Identification of a novel parahenipavirus-like contig***
130 During our extensive search for viruses in public databases, we detected a viral contig from
131 RNA-seq data derived from the kidney of *Blarina brevicauda* (family *Soricidae*, order
132 *Eulipotyphla*). A BLASTx search using this contig revealed that the top hit was the L protein of
133 Ninorex virus (WJL29506.1) [6], a member of the genus *Parahenipavirus*, with 74.6% amino
134 acid sequence identity (Table 1).
135 To evaluate the reliability of the contig, we mapped the RNA-seq data from which the
136 contig was derived to the viral contig sequence. Positions with a read depth of 5 or more were
137 considered reliable, and sequences that did not meet this threshold were removed. The final
138 contig length was 16,645 nucleotides. We named this virus Blarina brevicauda virus (BbV).
139

140 ***Annotation of the Blarina brevicauda virus genome***

141 Next, we determined the genome organization of BbV, which contains nine ORFs. Using the
142 amino acid sequences of these ORFs as queries, we performed BLASTp analysis and found
143 sequence similarity to the N, P/V/W/C, M, ORF X, F, G, and L genes of other parahenipaviruses
144 (Fig. 1a).

145 To identify the transcription signal sequences, we aligned the intergenic regions and
146 manually determined the sequences, referencing those of other orthoparamyxoviruses (Fig. 1b).
147 The intergenic CTT motif, conserved in most orthoparamyxoviruses, was also present in all
148 BbV intergenic regions. The putative transcription start and end signals were identified as
149 AGGAYHCARG and WTADRRARAA, respectively. Notably, the mapping data showed a
150 marked decrease in read depth in the intergenic regions (Figs. 1A and S1), suggesting active
151 viral transcription in the samples.

152 ORF X, located between the M and F genes, is a distinctive gene of parahenipaviruses and
153 is predicted to encode a transmembrane protein. Using DeepTMHMM, we predicted the
154 transmembrane region of BbV's ORF X to be between amino acids 39-60, which is similar to
155 other parahenipaviruses (Fig. 1C).

156 Paramyxoviruses typically utilize mRNA editing through the insertion of G nucleotide(s)
157 by transcriptional slippage to express P, V, and W proteins. To investigate whether BbV employs
158 the same mechanism, we reanalyzed the BbV mapping data. We detected G nucleotide
159 insertions at position 2,720 on the BbV genome (Figs. 1A and 1D), which result in the
160 production of three different proteins: the P protein (551 aa) without insertion, the V protein
161 (337 aa) with a single G insertion, and the W protein (281 aa) with two G insertions. Based on
162 the mapping data, the frequencies of expression for these proteins were 40.9%, 44.1%, and
163 15%, for P, V, and W, respectively (Fig. 1D and Supplementary Table 3).

164

165 ***Evolutionary analyses on Blarina brevicauda virus***

166 To elucidate the evolutionary relationship between BbV and other parahenipaviruses, we
167 performed phylogenetic analyses using the N and L proteins (Fig. 2A and B). In both
168 phylogenetic trees, BbV clustered with Ninorex virus, Wufeng Chodsigoa smithii henipavirus 1,
169 and a recently detected parahenipavirus LS-SCDW-1, all of which were detected in animals
170 from the family *Soricidae* (Fig. 3).
171 We then calculated amino acid sequence identities between BbV and its closest relatives,
172 Ninorex virus and Parahenipavirus sp. LS-SCDW-1, for the six major paramyxovirus proteins
173 (N, P, M, G, F, and L). The sequence identity for each protein is shown in Table 1; on average,
174 BbV shares 54.2% identity with Ninorex virus and 64.1% with Parahenipavirus sp. LS-SCDW-
175 1.
176
177 ***Parahenipaviruses may have largely evolved in co-divergence manner with the hosts***
178 To further understand the evolution of parahenipaviruses, we compared the topologies of trees
179 between parahenipaviruses and their hosts. Our analysis showed that the topology of the viral
180 phylogeny is largely congruent with that of the host phylogeny, although some incongruences
181 exist (Fig. 3) (see details in Discussion).
182
183 ***Comprehensive detection of BbV reads in public RNA-seq data***
184 To further characterize BbV, we conducted a comprehensive search for BbV-derived reads in
185 public RNA-seq data through mapping analysis. We mapped 306 RNA-seq datasets from
186 animals of the order *Eulipotyphla*, which includes *Blarina brevicauda*, the putative host of BbV,
187 to the viral contig and counted the mapped reads. As a result, BbV-derived reads were detected
188 in four additional RNA-seq datasets (Table 2), all of which belong to the same BioProject as the
189 one where BbV was initially identified. Among the five RNA-seq datasets positive for BbV
190 (including the initial dataset), four were derived from the kidney and one from the skin. The
191 number of viral reads in the kidney-derived datasets was markedly higher than in the skin-

192 derived dataset. Notably, while this BioProject also contains data from the brain, heart, liver,
193 and skin, no BbV-derived reads were detected in brain, heart, or liver; only one skin dataset
194 contained BbV reads (Table 2).
195
196 **Discussion**
197 Currently, 12 species and several unassigned viruses are reported in the genus *Parahenipavirus*
198 [1], but large phylogenetic gaps remain, and the diversity of parahenipaviruses has yet to be
199 elucidated. In this study, we identified the nearly complete genome sequence of a novel
200 paramyxovirus, BbV, from RNA-seq data of *Blarina brevicauda*. Notably, our analysis showed
201 that the average amino acid sequence identities of the six major viral proteins (N, P, M, F, G,
202 and L) between BbV and its closest relatives, Ninorex virus and parahenipavirus LS-SCDW-1,
203 are 54.2% and 64.1%, respectively. This meets the ICTV species demarcation criteria for the
204 family *Paramyxoviridae* (average identity below 85%;
205 https://ictv.global/ictv/proposals/2023.018M.Paramyxoviridae_reorg.zip [accessed on Sep 22,
206 2025]). Based on these findings, we propose that BbV is a novel species of virus within the
207 genus *Parahenipavirus*.
208 Our data strongly suggest that *B. brevicauda* is the authentic host of BbV. In metaviromic
209 analyses, viral contamination from various sources can sometimes occur, complicating the
210 identification of authentic hosts. However, our mapping analysis identified a significant number
211 of virus-derived reads in the kidney. Further, we showed a marked decrease in read depth in the
212 intergenic regions, suggesting that the virus actively transcribed in the samples. Additionally,
213 closely related parahenipaviruses have also been detected in shrews [2,3,5,6,20,21]. These
214 findings indicate that the virus is actively transcribed in the kidney, strongly suggesting that *B.*
215 *brevicauda* is the authentic host of BbV.
216 Parahenipaviruses may have largely evolved in co-divergence manner with the hosts. Our
217 analysis indicates that the topology of the viral tree is congruent with that of the host one, at

218 least at the genus level (Fig. 3). For viruses detected from *Crocidura* shrews, some
219 incongruences were observed, but this could be attributed to the difficulty of resolving
220 relationships within *Crocidura* because the diversification of those shrews seems to have
221 occurred over a very short timescale [22]. Therefore, our data suggest that parahenipaviruses
222 have largely co-diverged with their hosts. On the other hand, several parahenipaviruses have
223 been detected in multiple host species, indicating cross-species transmission. In particular, some
224 viruses within the lineage represented by Langya virus have been detected in species other than
225 shrews (including rodents and humans), suggesting cross-species transmission from shrews to
226 more distantly related animals. Note that evidence of cross-species transmission of
227 parahenipaviruses has so far been found in viruses in a limited lineage. Further identification of
228 parahenipaviruses filling the phylogenetic gap would provide insights into the evolution of
229 parahenipaviruses.

230 Our data also suggest that BbV is kidney-tropic. A total of 20 RNA-seq datasets from *B.*
231 *brevicauda* are registered under BioProject PRJNA788430, from which BbV was detected.
232 These datasets are derived from five different tissues (brain, skin, heart, kidney, and liver)
233 apparently from four individuals according to the metadata. Our mapping analysis revealed that
234 viral reads were almost exclusively found in the kidneys of all four individuals. Although viral
235 reads were detected in the skin of one individual, the quantity was significantly lower than in
236 the kidney. Thus, the viral reads in the skin sample might be due to contamination and/or index
237 hopping. Additionally, Hasua virus, Resua virus, Lechcodon virus, and Denwin virus,
238 parahenipaviruses detected from shrews, were suggested to be kidney-tropic [23]. Furthermore,
239 many parahenipaviruses were detected in the kidneys [2,5,6,24]. Thus, many of the members of
240 the genus *Parahenipavirus* may be kidney-tropic.

241 This study is the first to report the frequency of G-base insertions through RNA editing of
242 the P gene in parahenipaviruses. The transcripts encoding P, V, W proteins accounted for 40.9%,
243 44.1%, and 15% of the total on average, respectively (Fig. 1D). However, the ratio of P/V/W

transcripts is known to be changed depending on the infection stage [25,26]. Further analyses of infected animals and infection experiments will provide more detailed insights into the RNA-editing of parahenipaviruses.

During the preparation of this manuscript, an independent study reporting similar findings was published [20]. However, that study classified the virus within the genus *Henipavirus*, and therefore several taxonomic and related descriptions were inaccurate, as noted by Haring et al. [27]. Importantly, another paper also classified a parahenipavirus (isolate LS-SCDW-1; PQ421759.1) as a member of the genus *Henipavirus* [21]. In the present work, we analyze the virus as a member of the genus *Parahenipavirus*, providing a more accurate understanding of this virus.

In this study, we identified a novel paraphenipavirus from RNA-seq data derived from *B. brevicauda*, contributing to a deeper understanding of the diversity of parahenipavirus. However, significant phylogenetic gaps remain, and the characteristics of BbV are not yet fully understood. Further epidemiological and virological studies are necessary to better understand this virus.

Acknowledgments

The super-computing resources were provided by Human Genome Center, the Institute of Medical Science, the University of Tokyo and the NIG supercomputer at ROIS National Institute of Genetics.

This study was supported by KAKENHI grant numbers JP21H01199 (MH), JP22K19234 (MH), JP23K20902 (MH), JP24K21922(MH), and JP24K18455 (MK) and the 2024/2025 Osaka Metropolitan University (OMU) Strategic Research Promotion Project (Young Researcher) (MK).

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328 **Figure legends**

329 **Figure 1. Genome organization of Blarina brevicauda virus (BbV).**

330 **(A)** Schematic representation of the open reading frames (ORFs) and transcription signal
331 sequences of BbV. **(B)** Predicted transcription signal sequences of BbV. **(C)** Prediction of the
332 transmembrane domain of the ORF X protein using DeepTMHMM. **(D)** Schematic diagram
333 illustrating the P, V, and W proteins (left) and the average frequencies of guanine base insertions
334 (right). Detailed data are provided in Supplementary Table 3.

335

336 **Figure 2. Phylogenetic trees of parahenipaviruses, henipaviruses, and other**
337 **paramyxoviruses.**

338 Maximum likelihood phylogenetic trees were inferred using amino acid sequences of the N **(A)**
339 and L **(B)** proteins. Bootstrap values below 70 are not shown. The scale bar represents the
340 number of amino acid substitutions per site.

341

342 **Figure 3. Comparison of topologies between viral and host phylogenies.**

343 Phylogenetic trees of viruses (left) and hosts (right) are shown; the viral tree is adapted from
344 Fig. 2B, and the host tree was obtained from TimeTree 5 [28]. Blue lines link each virus to the
345 host species. Viruses lacking a corresponding host lineage in TimeTree 5 or detected in non-
346 shrew hosts are shown in gray.

347

Table 1. One-on-one BLASTp comparison between Blarina brevicauda virus and Ninorex virus

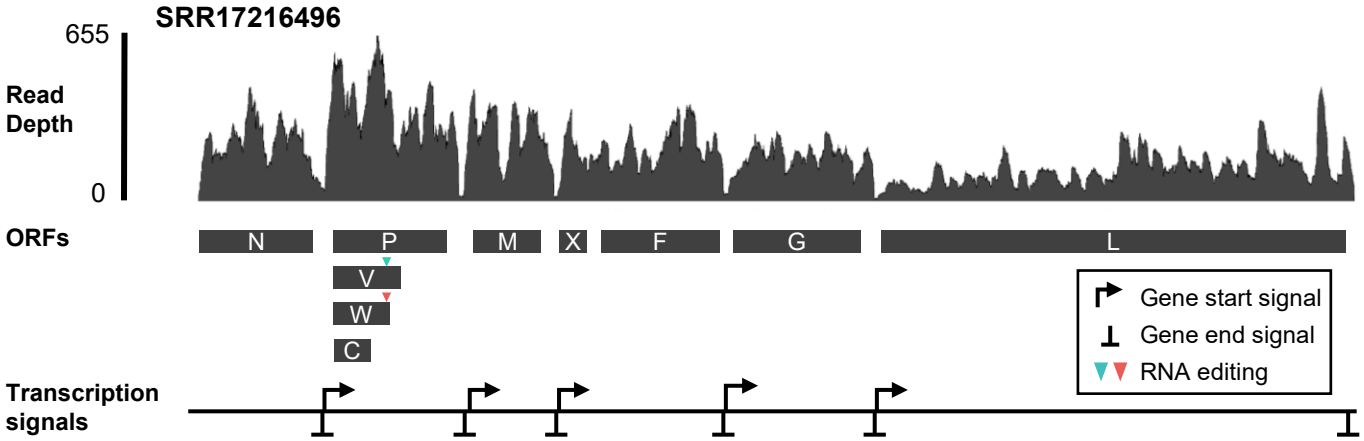
Protein	Identity (%)	
	Ninorex virus OQ438286.1	Parahenipavirus sp. LS-SCDW-1 PQ421759.1
N	50.7	73.8
P	34.7	44.6
M	76.2	79.4
F	55.7	62.4
G	33.4	48.6
L	74.6	75.8
Average	54.2	64.1

Table 2. Sample information and mapped read numbers in BioProject PRJNA788430

SRA Run accession	Sample name	Mapped read number	Read per million	Tissue
SRR17216479	Shrew217	0	0	Brain
SRR17216480		0	0	Heart
SRR17216481		14,304	124.2	Kidney
SRR17216482		0	0	Liver
SRR17216483		0	0	Skin
SRR17216484	Shrew218	0	0	Brain
SRR17216485		0	0	Heart
SRR17216486		7,081	51.89	Kidney
SRR17216487		0	0	Liver
SRR17216488		0	0	Skin
SRR17216489	Shrew220	0	0	Brain
SRR17216490		0	0	Heart
SRR17216491		18,978	170.74	Kidney
SRR17216492		0	0	Liver
SRR17216493		35	0.26	Skin
SRR17216494	Shrew221	0	0	Brain
SRR17216495		0	0	Heart
SRR17216496		24,085	186.08	Kidney
SRR17216497		0	0	Liver
SRR17216498		0	0	Skin

Figure 1

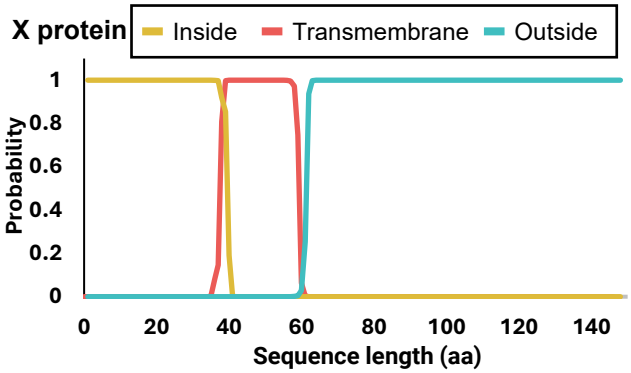
A



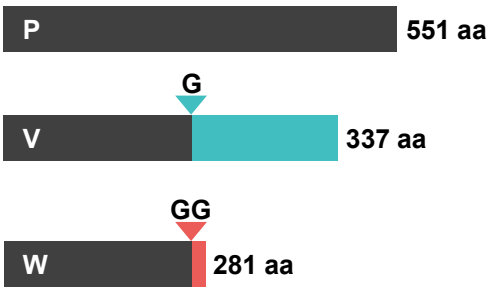
B

	Gene end	Intergenic region	Gene start
N-P	TAATAAAAAA	CTT	AGGATCCAGG
P-M	TTAGAAAAAA	CTT	AGGATTCAAG
M-X/F	TTAAAGAAAA	CTT	AGGATTCAAG
X/F-G	TTATAAAAAA	CTT	AGGATTCAAG
G-L	ATATGAAGAA	CTT	AGGACACAAG
L-trailer	TTAAGAAAAA		

c



d



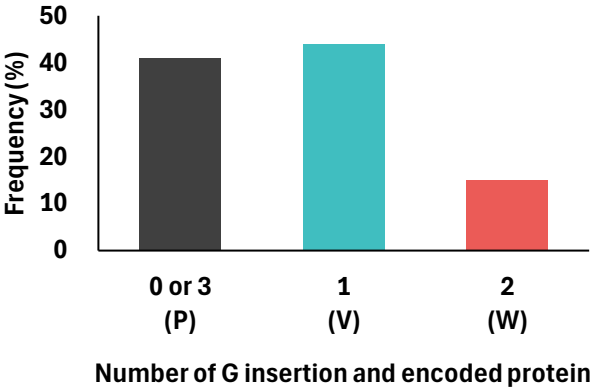
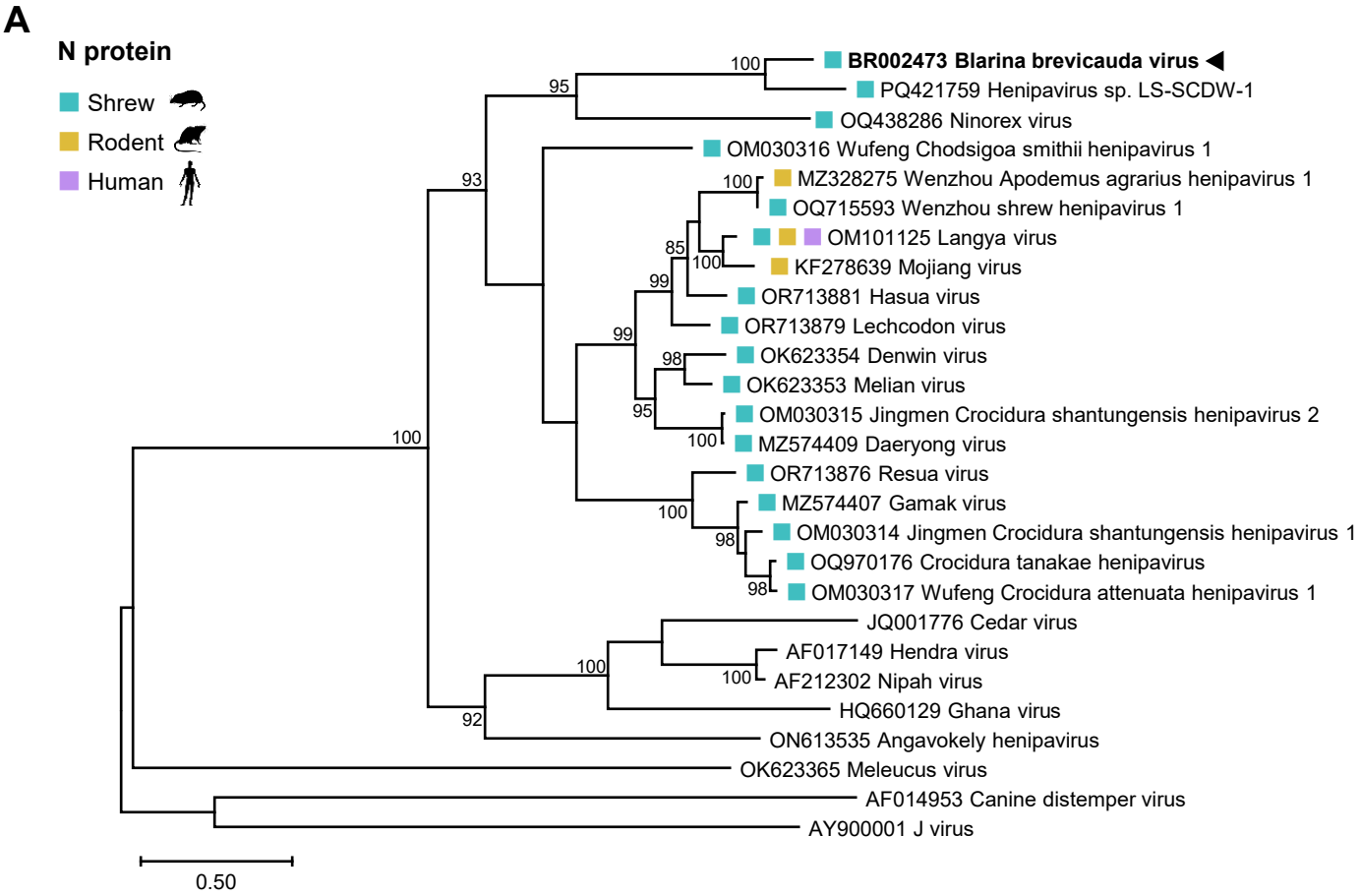


Figure 2


A
N protein
Shrew
Rodent
Human
BR002473 Blarina brevicauda virus
PQ421759 Henipavirus sp. LS-SCDW-1
OQ438286 Ninorex virus
OM030316 Wufeng Chodsigoa smithii henipavirus 1
MZ328275 Wenzhou Apodemus agrarius henipavirus 1
OQ715593 Wenzhou shrew henipavirus 1
OM101125 Langya virus
KF278639 Mojiang virus
OR713881 Hasua virus
OR713879 Lechcodon virus
OK623354 Denwin virus
OK623353 Melian virus
OM030315 Jingmen Crocidura shantungensis henipavirus 2
MZ574409 Daeryong virus
OR713876 Resua virus
MZ574407 Gamak virus
OM030314 Jingmen Crocidura shantungensis henipavirus 1
OQ970176 Crocidura tanakae henipavirus
OM030317 Wufeng Crocidura attenuata henipavirus 1
JQ001776 Cedar virus
AF017149 Hendra virus
AF212302 Nipah virus
HQ660129 Ghana virus
ON613535 Angavokely henipavirus
OK623365 Meleucus virus
AF014953 Canine distemper virus
AY900001 J virus
0.50

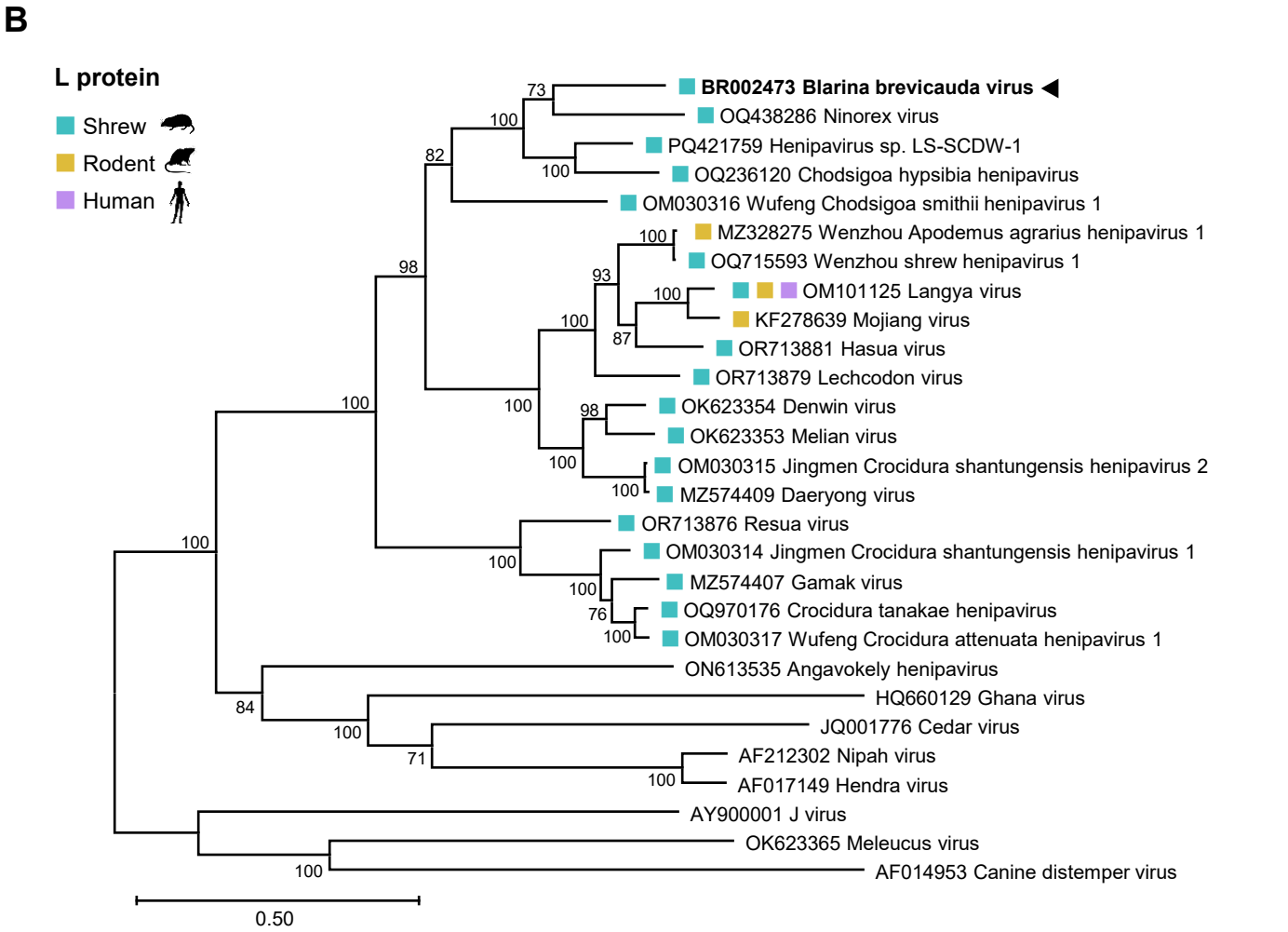
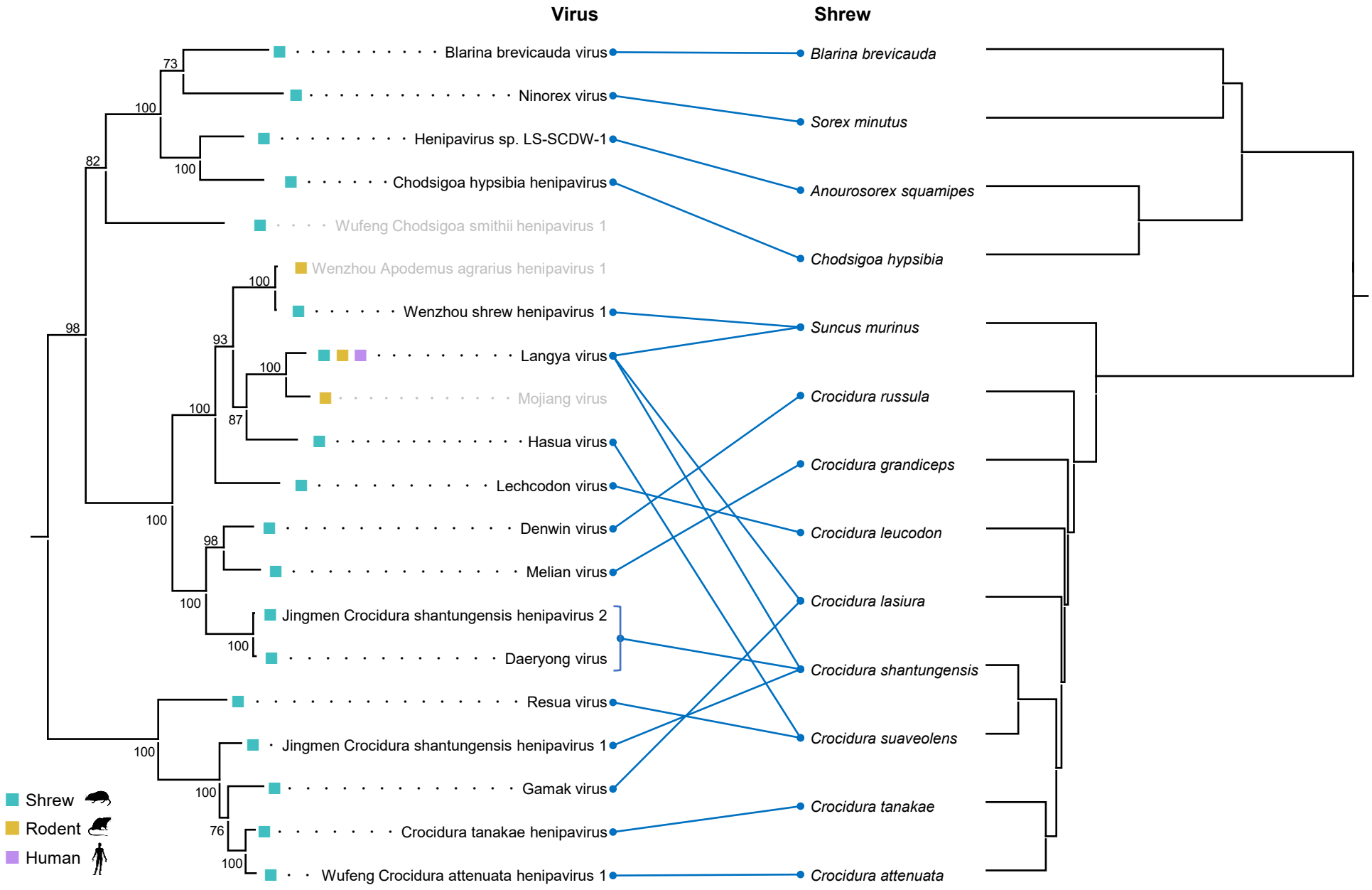

B
L protein
Shrew
Rodent
Human
BR002473 Blarina brevicauda virus
OQ438286 Ninorex virus
PQ421759 Henipavirus sp. LS-SCDW-1
OQ236120 Chodsigoa hypsibia henipavirus
OM030316 Wufeng Chodsigoa smithii henipavirus 1
MZ328275 Wenzhou Apodemus agrarius henipavirus 1
OQ715593 Wenzhou shrew henipavirus 1
OM101125 Langya virus
KF278639 Mojiang virus
OR713881 Hasua virus
OR713879 Lechcodon virus
OK623354 Denwin virus
OK623353 Melian virus
OM030315 Jingmen Crocidura shantungensis henipavirus 2
MZ574409 Daeryong virus
OR713876 Resua virus
OM030314 Jingmen Crocidura shantungensis henipavirus 1
MZ574407 Gamak virus
OQ970176 Crocidura tanakae henipavirus
OM030317 Wufeng Crocidura attenuata henipavirus 1
ON613535 Angavokely henipavirus
HQ660129 Ghana virus
JQ001776 Cedar virus
AF212302 Nipah virus
AF017149 Hendra virus
AY900001 J virus
OK623365 Meleucus virus
AF014953 Canine distemper virus
0.50

Figure 3


Virus
Shrew
Blarina brevicauda virus
Ninorex virus
Henipavirus sp. LS-SCDW-1
Chodsigoa hypsibia henipavirus
Wufeng Chodsigoa smithii henipavirus 1
Wenzhou Apodemus agrarius henipavirus 1
Wenzhou shrew henipavirus 1
Langya virus
Mojiang virus
Hasua virus
Lechcodon virus
Denwin virus
Melian virus
Jingmen Crocidura shantungensis henipavirus 2
Daeryong virus
Resua virus
Jingmen Crocidura shantungensis henipavirus 1
Gamak virus
Crocidura tanakae henipavirus
Wufeng Crocidura attenuata henipavirus 1
Blarina brevicauda
Sorex minutus
Anourosorex squamipes
Chodsigoa hypsibia
Suncus murinus
Crocidura russula
Crocidura grandiceps
Crocidura leucodon
Crocidura lasiura
Crocidura shantungensis
Crocidura suaveolens
Crocidura tanakae
Crocidura attenuata
73
100
82
100
100
93
100
87
98
100
100
98
100
100
100
100
76
100
Shrew
Rodent
Human