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A novel bocavirus from domestic mink, China

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Abstract Bocaviruses have been found in the feces of humans and a variety of animals, including pigs, cattle, dogs, gorillas, cats, and sea lions. Here, we have characterized the almost complete genome (5224 nt) of a novel bocavirus from feces of domestic minks, which has been provisionally named mink bocavirus. The NS1 protein of mink bocavirus shared 36.9–52 % amino acid sequence identities with those of other known bocaviruses and phylogenetically clustered with bocaviruses from other carnivores. According to the genetic distance-based criteria, mink bocavirus qualifies as a novel species of bocavirus. PCR of feces from a group of domestic minks, which included both healthy animals and animals suffering from diarrhea, revealed that 30 % (9/30) shed virus. However, no association between viral shedding and the presence of diarrhea could be determined.

Keywords Mink · Viral metagenomics · Bocavirus · Phylogenetic analysis · Genome structure

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Parvoviruses are small non-enveloped icosahedral viruses with a linear single-strand DNA genome of approximately 5 kb. The family *Parvoviridae* consists of two subfamilies. Members of the *Densovirinae* are known to infect insects, while those of the *Parvovirinae* infect vertebrates. The first characterized bocavirus, a member of the *Bocaparvovirus* genus, one of eight currently recognized genera in the *Parvovirinae*, was initially isolated in 1961, and subsequently multiple other bocaviruses have been detected in various animals and humans [1–7]. Human bocavirus is thought to cause respiratory tract infections [8], but whether bocaviruses can cause disease in animals is unknown [4].

In order to investigate the intestinal virome of domestic minks, 30 fecal samples from 10 diarrheal and 20 healthy animals were collected in 2014 from the Jinzhou economic animal breeding center. Samples were immediately stored at −80 °C and shipped on dry ice.

Viral metagenomics was used to characterize viral sequences [9–12]. Fecal samples were suspended in Dulbecco's Phosphate-Buffered Saline (DPBS), vortexed for 10 min, and then centrifuged at 13,400 $\times g$ for 10 min. The stool suspensions were collected in 1.5 ml centrifuge tubes. Three pools were randomly generated, each of which contained 10 fecal suspensions. After low-speed centrifugation at 5000 $\times g$ and filtration, the samples were treated with DNase (Turbo DNase from Ambion, Baseline-ZERO from Epicentre, and Benzonase from Novagen) and RNase (Fermentas) to reduce levels of mink nucleic acids, while viral genomes remain protected within the viral capsid [13]. Three libraries were then constructed using the Nextera XT DNA Sample Preparation Kit (Illumina) and sequenced using the MiSeq Illumina platform with 250 bases paired ends with a distinct molecular tag for each pool. Resulting raw reads were trimmed for quality and

primer, and *de novo* assembled into contigs. Sequences and contigs were compared to the GenBank non-redundant protein database using BLASTx with an *E*-value cutoff of $<10^{-5}$.

Results showed that 81 sequence reads showed similarity to known canine bocavirus with an *E*-value cutoff of $<10^{-5}$, including a large contig of 4134 bp, a small contig of 549 bp, and a total of 79 unassembled singlet sequences. To obtain the

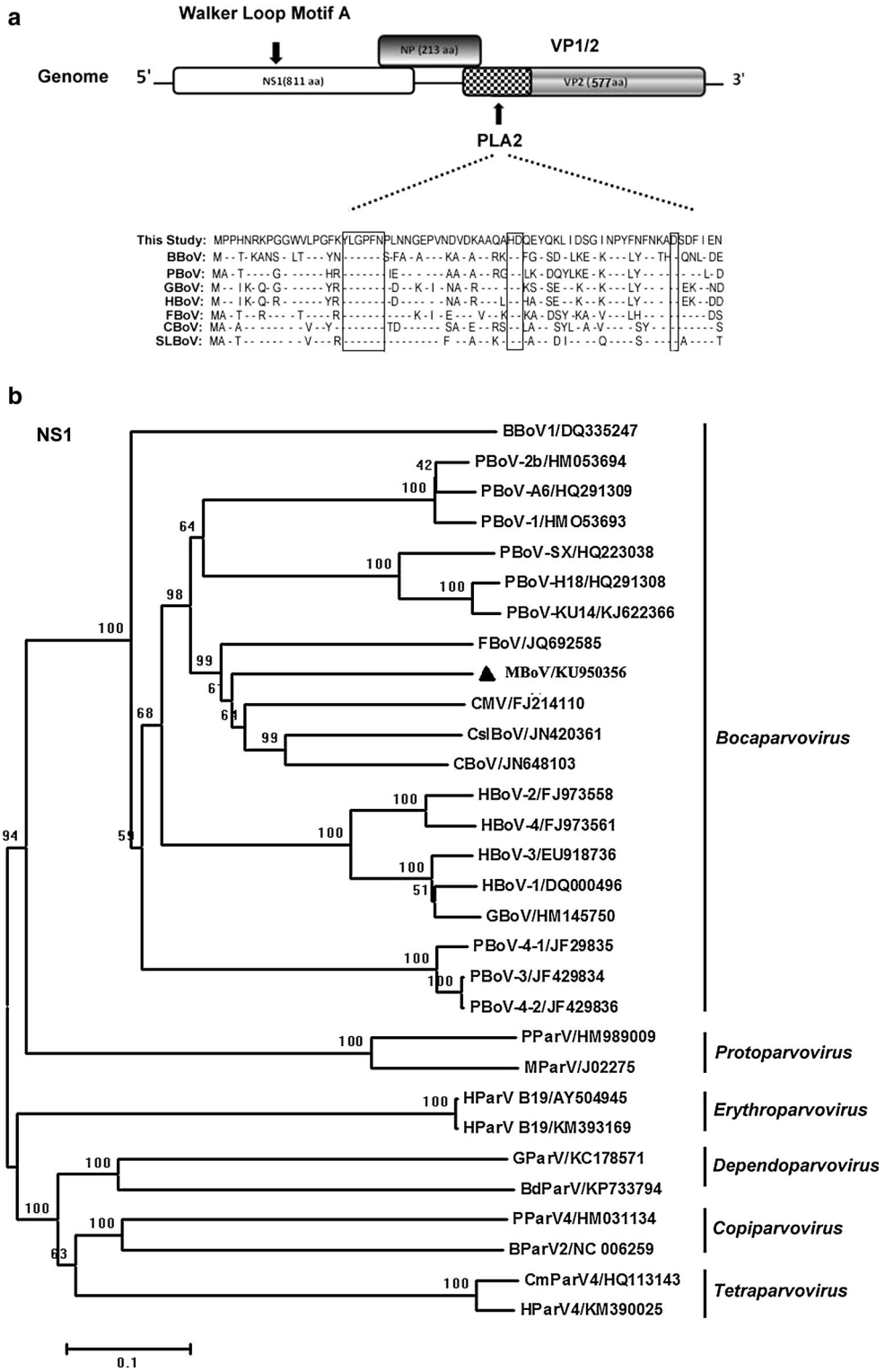

Fig. 1 The genome and phylogeny of mink bocavirus. **a** Organization of the mink bocavirus genome. Four encoding proteins (NS1, NP1, VP1, and VP2) are shown. The conserved ATP-binding Walker loop motif and the PLA2 similarity region, including the calcium-binding region and catalytic resides are shown. **b** Phylogenetic analyses of the amino acid sequences of bocavirus NS1 protein of different genotypes and species. Representative strains and GenBank accession numbers of bocaviruses are shown. *Scale bar* indicates nucleotide substitutions per site. *Bootstrap values* (based on 1000 replicates) for each node are given

complete open reading frames, PCR primers were designed to bridge sequence gaps and amplicons were Sanger sequenced. The virus was named mink bocavirus and submitted to GenBank with accession no. KU950356. The partial genome of mink bocavirus was 5224 nucleotides with the Untranslated Regions (UTRs) located in position 1–75 and position 5085–5224, and had a base composition of 31.45 % A, 24.41 % G, 24.96 % T, and 19.18 % C. Four ORFs were predicted using NCBI's ORF Finder (Fig. 1a). The NS1 protein encoded by ORF1 was 811 aa long, with conserved motifs associated with rolling circle replication, helicase, and ATP-bing Walker loop motif (^{467}GPASTGKT^{474}) (Fig. 1a), which is the second longest NS protein encoded by a bocavirus, being shorter than that of bovine parvovirus (860 aa). ORF2 and ORF3 encoded two structural proteins, VP1 and VP2, respectively. The length of VP1 was 716 aa. In the N-termini of VP1, phospholipase A2 motifs (PLA2) with the calcium-binding loop and phospholipase catalytic residues (Fig. 1a) are present. VP2 gene completely overlapped with the VP1, encoding 577 aa, of which a glycine-rich sequence was present in the N terminus. The middle ORF4 was 642 nt in length encoding the 213 aa NP1 protein.

To determine the relationship between this mink bocavirus and the other members of the *Bocaparvovirus* genus, phylogenetic analysis based on the NS1 amino acid sequences was performed. Amino acid sequences were aligned by CLUSTAL W, and phylogenetic trees were constructed using molecular evolutionary genetics analysis (MEGA) v6 software in neighbor-joining method mode with 1000 bootstrap replicates. Result showed that mink bocavirus clustered with feline bocavirus (FBoV, JQ692585), canine minute virus (CMV, FJ214110), California sea lion bocavirus 1 (CslBoV, JN420361), and canine bocavirus 1 (CBoV, JN648103) formed a distinct clade (Fig. 1b). Comparing mink bocavirus with human BoVs, porcine BoVs, gorillas BoVs, feline BoVs, canine BoVs, and sea lion BoVS, the amino acid sequence identities of NS1, VP1, VP2, and NP1 were 36.9–52 %, 41.1–63 %, 40.8–62 %, and 31–50 %, respectively. The NS1 protein of mink bocavirus shared the highest amino acid sequence identity (52 %) and nucleotide sequence identity (58.5 %) with that of feline bocavirus strain (HK797F/JQ692585). The International Committee on Taxonomy of Viruses criteria for classification of parvovirus specify that a new parvovirus species should have <85 % identity of the NS1 protein compared to other known parvovirus species. The mink bocavirus in this study has between 36.9 and 52 % amino acid identity in the NS1 protein compared to other known bocavirus species, indicating that mink bocavirus belongs to a novel bocavirus species.

In order to investigate the prevalence of mink bocavirus in domestic minks, viral DNA genome was extracted from all 30 individual fecal samples using TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (TaKaRa, Japan). A set of nested primers were designed based on the NS1 nucleotide sequence of mink bocavirus to perform PCR screening. Primers TCBOVWS (5′-TCCAGAGAGC GCTCGGCAGT-3′) and TCBOVWX (5′-GCGGCGTC GCCAATTCTGGA-3′) were used for the first round of PCR, and TCBOVNS (5′-CCGGGACCGGATGAGCC TGA-3′) and TCBOVNX (5′-GCAACTGACGCGTTCAG CTTCAAA-3′) for the second round. The expected length of amplified fragment was 378 bp. Results indicated that 9 (30 %, 9/30) samples were positive for mink bocavirus, among which four were from minks with diarrhea, while the other five were from healthy minks. The specific PCR products were sequenced using the Sanger method. Sequencing results generated five different sequences which were submitted to GenBank with accession nos. KX644860-KX644864. Sequence analysis based on the 378 bp sequences indicated that the nine mink bocaviruses shared >98 % nucleotide identity over the NS1 region sequenced, indicating low viral diversity in this cohort of domestic minks.

Taken together, we have identified a novel bocavirus in mink and almost completely characterized its genome. Phylogenetic analysis indicated that mink bocavirus clustered with FBoV, CMV, CslBoV, and CBoV from other carnivores. Comparing the NS1 protein of mink bocavirus with that of its closest feline bocavirus, the identity was only 52 %, suggesting that mink bocavirus represents a novel species of Bocavirus.

An epidemiological study suggested that a single strain of mink bocavirus was prevalent in domestic minks from the Jinzhou economic animal breeding center. Mink bocavirus could be PCR amplified in fecal samples from both diarrheal and healthy minks, indicating that detection of this virus in feces was not associated with diarrhea.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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