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LETTER

A novel mosquito-borne reassortant orbivirus isolated from Xishuangbanna, China

Dear Editor,

The genus *Orbivirus*, within the family *Reoviridae*, includes 22 virus species (King et al., 2011). They are distributed globally, but are particularly prevalent in Europe, Asia, and Africa. In addition, they can be transmitted by ticks or other hematophagous insect vectors, including *Culicoides*, mosquitoes, and sandflies (Belaganahalli et al., 2015).

Orbiviruses contain double-stranded RNA as their genetic material, which includes 10 segments (S1–S10) of various lengths. Owing to this segmented structure, genetic reassortment occurs frequently among orbiviruses. Reassortment occurs when different segmented viruses of the same vector type infect a single host cell, and genomic segments of the "parental" viruses are exchanged and repackaged during progeny formation (Simon-loriere and Holmes, 2011). Reassortment can result in novel viral genotypes and subsequent phenotypes, which can alter the mechanism of immune escape, the potential range of hosts or vectors, and the mechanism of virulence or pathogenicity (Mcdonald and Patton, 2011). In general, reassortment represents a critical mechanism in the evolution of segmented viruses. Accordingly, to understand viral diversity, it is important to identify and characterize reassortant taxa.

To identify novel reassortant orbiviruses, specimens were collected during the active mosquito season (from June to September) in Xishuangbanna, Yunnan Province in southwestern China in 2007. A sample was virus-positive if it had a cytopathic effect in three successive cell passages (Zuo et al., 2014). Supernatants containing the virus were identified by reverse transcription-polymerase chain reaction (RT-PCR) with genus- or species-specific primers designed for potential mosquito-borne viruses (Lei et al., 2014). Virus-containing samples that could not be identified by RT-PCR were identified by high-throughput sequencing (HTS) (Patel and Jain, 2012). Complete nucleotide sequences for segments 1–10 (Seg1–10) of a virus, referred to as *Banna orbivirus* (BAOV), were determined. The sequences have been submitted to GenBank, with sequential accession numbers KX455487–KX455496. The full-length sequence genome map and the protein sequence of the 10 BAOV segments are shown in Figure 1A.

Recently, several novel orbiviruses have been isolated. Among the most common orbiviruses, *Tibet orbivirus* (TIBOV) (XZ0906) was first isolated from a pool of *Anopheles maculatus* mosquitoes in Tibet, China in 2009. TIBOV (YN12246) was isolated from *Culicoides* spp. in Yunnan, China, and *Fengkai orbivirus*, which also belongs to TIBOV, was isolated from a pool of *Culex fatigan* mosquitoes in Guangdong Province, China in 2008. A comparison of gene and protein sequences of BAOV segments to those of *Fengkai orbivirus* and XZ0906 is summarized in Table 1. The complete genomes of BAOV, *Fengkai orbivirus*, and XZ0906 were 19270, 19349, and 19235 bp, respectively, and had similar GC contents–42.4%, 42.7%, and 42.5%, respectively. Each genomic segment encodes a single major open reading frame. S1–10 encode seven structural proteins: RNA-dependent RNA polymerase (RdRp), outer capsid protein one (OC1), inner sub-core shell 'T2' protein, Cap, Tup, Hel, and OC2, and three non-structural proteins (NS), NS2, NS4, and NS3 (Moss et al., 1992).

To determine the phylogenetic relationships among BAOV and other viruses in the genus *Orbivirus*, multiple sequence alignments were generated using Mega v6.06 with the complete sequences of BAOV and 15 other orbiviruses (Tamura et al., 2013) (Supplementary Table S1). Phylogenetic trees based on all 10 viral genome segments were constructed from the nucleotide sequence alignments by using the maximum likelihood method with the GTR+G+I model. The robustness of the tree was established by a bootstrap analysis with 1, 000 replicates. To determine similarity, Simplot 3.5 (http://www.med.jhu.edu/deptmed/sray/download/; provided by S. Ray) was used to generate similarity plots based on the Cluster alignment (Moss et al., 1992). To better understand the genetic relationships among BAOV and other orbiviruses, 10 phylogenetic trees were constructed based on S1–10 (Supplementary Figure 1A–1J). BAOV, *Fengkai orbivirus*, and XZ0906 were grouped into a single cluster, which was distinct from other orbiviruses. S3 from BAOV had a higher amino acid sequence homology with S3 from YN12246 (94%) than with S3 from

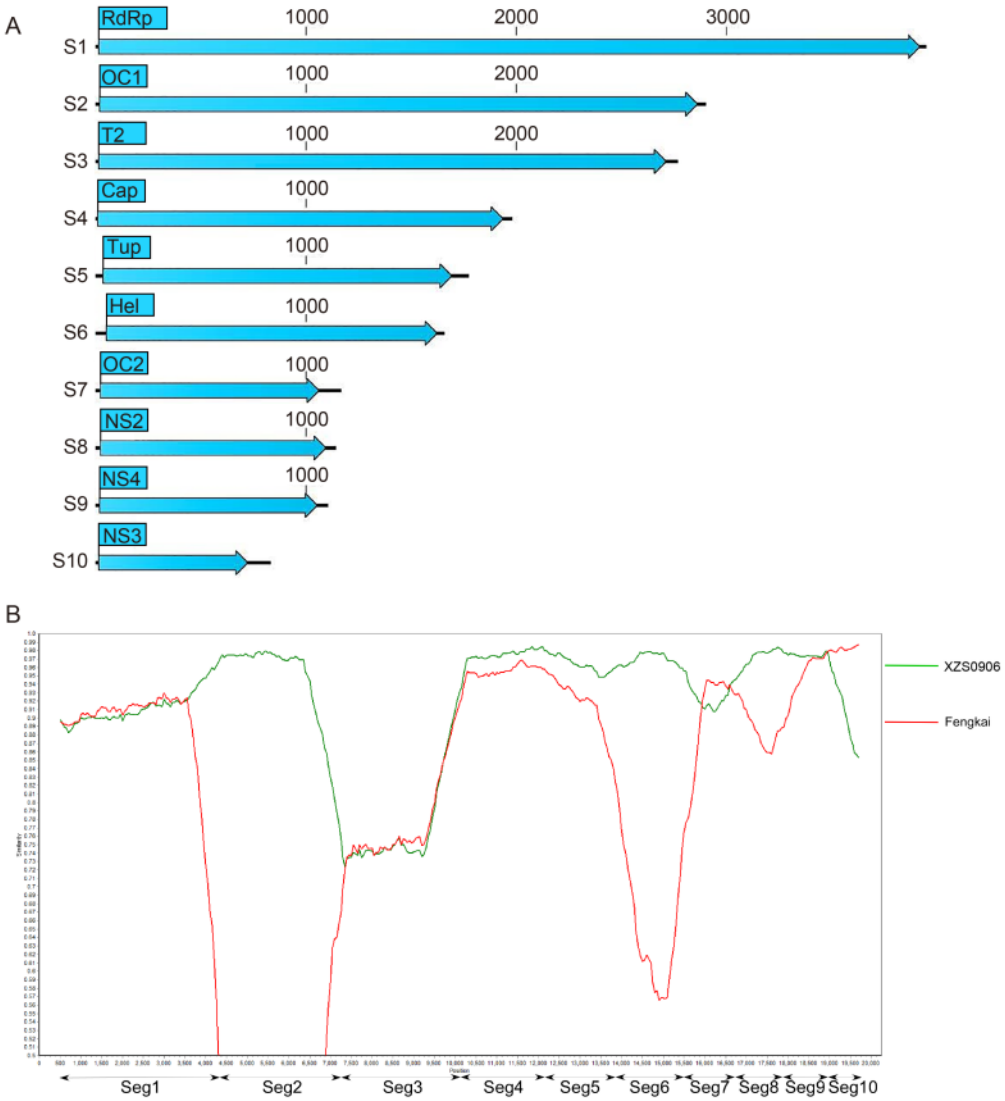

Figure1. (A) The full-length sequence genome map and protein sequence of 10 BAOV segments. (B) Similarity among BAOV, *Fengkai orbivirus*, and XZ0906. The red line represents TIBOV (XZ0906); the green line represents *Fengkai orbivirus*; the horizontal axis indicates the degree of sequence similarity; the vertical axis indicates the position of genomic S1–10 sequences.

Fengkai orbivirus (80%) or S3 from XZ0906 (80%). However, sequences for only two segments (S1 and S3) from YN12246 are available in the GenBank database. The remaining BAOV segments had high identities to those of Fengkai orbivirus and XZ0906. S2 and S6 from BAOV exhibited high sequence similarities with S2 and S6 from *Fengkai orbivirus*. S10 from BAOV exhibited high sequence similarity with S10 from XZ0906 (Supplementary Figure S1). The degrees of nucleotide and amino acid sequence homology between the 10 segments in BAOV and those of other orbiviruses were also examined using BLASTn and BLASTp, respectively (Supplementary Table S2). S2 and S6 from BAOV had higher nucleotide and amino acid sequence homology with S2 and S6 from *Fengkai orbivirus* (97% and 98%) than with S2 and S6 from *Tibet orbivirus* (70% and 71%). S10 from BAOV had higher nucleotide and amino acid sequence homology with S10 from XZ0906 (99%) than with S10 from *Fengkai orbivirus* (85%). Simplot and Bootscan analyses revealed the similarity among BAOV, *Fengkai orbivirus*, and XZ0906 genomic sequences in each region (Figure 1B). These results are consistent with those of the BLASTn analysis, as shown in Supplementary Table S2. S2 and S6 from BAOV were more similar to S2 and S6 from *Fengkai orbivirus* than to S2 and S6 from XZ0906. S10 from BAOV was more

Table 1. Characteristics of dsRNA genome segments and proteins of BAOV, *Fengkai orbivirus*, and XZ0906

Virus/ segment	Segment length (bp)	Protein encoded	Predicted protein length (aa)	ORFs (bp, including stop codon)	5′ NCRs	3′ NCRs	%GC Content	Accession No.
BAOV								
Seg1	3950	RdRp	1326	12-3926	11	23	41.1	KX455487
Seg2	2901	OC1	966	13-2865	12	35	39.8	KX455488
Seg3	2769	T2	916	11-2716	10	52	43.8	KX455489
Seg4	1978	Cap	654	9-1940	8	37	42.9	KX455490
Seg5	1775	TuP	546	83-1696	82	78	44.3	KX455491
Seg6	1656	Hel	505	47-1626	46	29	45.2	KX455492
Seg7	1165	OC2	355	18-1067	17	97	41.9	KX455493
Seg8	1142	NS2	365	21-1100	20	31	40.9	KX455494
Seg9	1102	NS4	352	15-1057	14	44	42.4	KX455495
Seg10	832	NS3	241	13-726	12	105	44.9	KX455496
Total	19270						42.4	KX455497
Fengkai								
Seg1	3950	RdRp	1326	12-3926	11	23	40.5	NC_027803.1
Seg2	2999	OC1	941	14-2791	13	207	40.0	NC_027804.1
Seg3	2761	T2	914	18-2717	17	43	44.0	NC_027805.1
Seg4	1979	Cap	654	9-1940	8	38	43.0	NC_027806.1
Seg5	1775	TuP	563	32-1696	31	78	43.1	NC_027807.1
Seg6	1636	Hel	535	27-1607	26	28	42.0	NC_027811.1
Seg7	1165	OC2	355	18-1067	17	97	45.0	NC_027812.1
Seg8	1151	NS2	368	21-1109	20	41	42.0	NC_027813.1
Seg9	1100	NS4	352	15-1055	14	44	44.2	NC_027814.1
Seg10	833	NS3	238	22-726	21	106	41.5	NC_027815.1
Total	19349						42.7	
XZ0906								
Seg1	3950	RdRp	1326	12-3926	11	23	40.8	KF746187.1
Seg2	2888	OC1	962	14-2854	13	33	40.7	KF746188.1
Seg3	2769	T2	914	18-2717	17	51	44.2	KF746189.1
Seg4	1978	CaP	654	9-1940	8	37	42.8	KF746190.1
Seg5	1775	TuP	563	32-1696	31	78	43.4	KF746191.1
Seg6	1636	Hel	535	27-1607	26	28	42.1	KF746192.1
Seg7	1165	OC2	355	18-1067	17	97	45.3	KF746193.1
Seg8	1142	NS2	365	21-1100	20	41	43.6	KF746194.1
Seg9	1100	NS4	352	15-1055	14	44	44.6	KF746195.1
Seg10	832	NS3	238	22-726	21	105	41.3	KF746196.1
Total	19235						42.5	

similar to S10 from XZ0906 than to S10 from *Fengkai orbivirus*.

Reassortment is a common event in the evolution of viruses. In this study, we report a novel mosquito-borne reassortant *Orbivirus* species, which we named *Banna orbivirus* (BAOV). The virus was isolated from a *Culex*

tritaeniorhynchus pool collected in Xishuangbanna, Yunnan Province, China in 2007. Whole genome and phylogenetic analyses revealed that BAOV is a reassortant of several *Tibet orbivirus* strains. It has a double-stranded RNA genome of 19, 270 bp, containing ten segments (S1–S10) of various lengths. The 10 segments of BAOV had high nucleotide and amino acid sequence similarity with segments of either *Fengkai orbivirus* or *Tibet orbivirus* (TIBOV, XZ0906 strain). Phylogenetic analyses indicated that the novel BAOV is a reassortant derived from XZ0906 and *Fengkai orbiviruses*, and is a *Tibet orbivirus*. This is the first report of a novel genomic reassortment of *Tibet orbivirus* isolated in China; these findings contribute to our understanding of the diversity of orbiviruses.

FOOTNOTES

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Supplementary figure/tables are available on the websites of *Virologica Sinica*: www.virosin.org; link.springer.com/journal/12250.

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REFERENCES

Belaganahalli MN, Maan S, Maan NS, et al. 2015. Viruses, 7: 2185–2209.
Herniou EA, Arif BM, Becnel JJ, et al. 2012. Family Reoviridae. In: Virus Taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. King AMQ, Adams MJ, Cartens EB, Lefkowitz EJ (eds). Amsterdam: Elsevier, pp. 855–881.
Lei W, Guo X, Fu S, et al. 2014. Plos One, 10: e0136257.
Mcdonald SM, Patton JT. 2011. Trends Microbiol, 19: 136–144.
Moss SR, Jones LD, Nuttall PA. 1992. J Gen Virol, 73: 2585–2590.
Patel RK, Jain M. 2012. Plos One, 7: e30619.
Simon-Loriere E, Holmes EC. 2011. Nat Rev Microbiol, 9: 617–626.
Tamura K, Stecher G, Peterson D, et al. 2013. Mol Biol Evol, 30: 2725–2729.
Zuo S, Zhao Q, Guo X, et al. 2014. Virus Res, 180: 31–38.