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Two putative novel serotypes of Tibet orbivirus isolated from *Culicoides* spp. in Yunnan, China

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ABSTRACT

Tibet orbivirus (TIBOV) was identified as a novel orbivirus in 2014. Antibodies against TIBOV were detected in cattle, Asian buffalo, and goats, while all the sequenced TIBOV strains were isolated from mosquitos and *Culicoides*. The known TIBOV strains have been classified into four putative serotypes. In this study, two TIBOV strains isolated from *Culicoides* spp. in Shizong County of Yunnan Province, China, were fully sequenced. The phylogenetic analysis of outer capsid protein 2 (VP2) indicated that these two viral strains belong to two novel putative serotypes of TIBOV. The updated putative serotypes may help in an investigation of the distribution and virulence of TIBOV.

Keywords: Tibet orbivirus; TIBOV; serotype; *Culicoides*

INTRODUCTION

Twenty-two virus species are currently recognized by the International Committee on Taxonomy of Viruses (ICTV) as members of the genus *Orbivirus* (family *Sedoreoviridae*) [1]. Well-known *Orbivirus,* bluetongue virus (BTV), and epizootic hemorrhagic disease virus (EHDV) have caused some outbreaks of diseases in ruminants, such as cattle, sheep, and deer [2]. The African horse sickness virus (AHSV) recently caused a serious epidemic in horses in Thailand [3]. A virus isolated from mosquitoes in Tibet, China, in 2009 was identified as a novel orbivirus and called Tibet orbivirus (TIBOV) by Li et al. [4]. Eight TIBOV strains isolated from mosquitoes and *Culicoides* spp. were reported and seven of them were completely sequenced [4-9]. On the other hand, no TIBOV has been isolated from mammals, but antibodies against TIBOV were detected in the serum samples of cattle, Asian buffalo, and goats [6]. No serum neutralization experiment was performed to identify the serotypes, but phylogenetic analysis suggested that these TIBOV genomes represent several putative serotypes [9].

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Conflict of Interest

The authors declare no conflicts of interest.

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MATERIALS AND METHODS

Viral isolation and sequencing

In this study, two strains of TIBOV (YNV/KM-1 and YNV/17-14) were isolated from *Culicoides* spp. at Wulong Village (24.64°N, 104.29°E) in Shizong County of Yunnan Province, China, in 2019 and 2020, respectively, were completely sequenced. Briefly, viral isolation was performed as described by Duan et al. [10]. The viral genomic dsRNAs were extracted from isolate-infected BHK-21 cells [10] and amplified using a "Full-Length Amplification of cDNAs" (FLAC) method [11]. The DNA products were sequenced by MAGIGEN Company (China) using a HiSeq 2000 system, followed by reads preparation, reads/sequences checking, and contigs assembling using the Soapnuke (v2.0.5), BWA (v0.7.17) and Megahit (v1.1.2) software, respectively.

Electron micrograph of virions

Viral strain infected monolayer BHK-21 (225 cm^2) showing cytopathic effects (CPEs) was frozen and thawed twice, and the cellular debris was removed through centrifugation at 3,500 rpm for 30 min. The supernatant was centrifuged at 40,000 rpm for 4 h. Virions deposits were suspended by 100 μL PBS, and a drop was placed onto a copper screen with a Formver membrane. The sample was stained with 2% phosphotungstic acid for 1.5 min, and then air dried. Photographs of virions were taken by transmission electron microscopy (TEM; Thermo Scientific, USA). Viral diameters were measured by Photoshop (2020) based on the photos. Median values and 95% confidence intervals (CIs) of diameters were calculated by PASW Statistics (version 18; SPSS Inc., USA).

Phylogenetic analysis

For phylogenetic analysis, the genomic sequences of the seven published TIBOV strains (**Supplementary Table 1**) and representative strains from 20 species of the *Orbivirus* genus, as recognized by the ICTV (**Supplementary Table 2**), were obtained from GenBank. MEGA-11 software was used to perform sequence alignments and construct phylogenetic trees. Complete coding sequences (CDS) were aligned using the muscle (codon) algorithm with default parameters, while the phylogenetic trees were constructed using the Neighbor-Joining (NJ) algorithm (bootstrap = 1,000; Model = Kimura 2; d: Transitions + Transversions; Codon position = 1). Genetic distances were calculated manually according to the branch lengths of the phylogenetic tree.

RESULTS

Virions in the supernatants of strains infected BHK-21 cells were observed by TEM. Spherical viral particles without an envelope were noted (**Fig. 1**). The viral diameters of the strain YNV/KM-1 and YNV/17-14 were estimated to be 70.9 nm (95% CI, 68.2–72.2 nm; n = 10) and 74.7 nm (95% CI, 68.5–76.5 nm; n = 5), respectively, according to the photographs (**Fig. 1**).

Table 1 lists the data of the novel strains, and **Supplementary Table 3** provides more details. All the segments possessed an identical six-based untranslated sequence at the 5' end (5-GUAAAA) and 3' end (ACUUAC-3), respectively (**Supplementary Table 3**). Seg2 and Seg6, which encoded the outer capsid proteins VP2 and VP5, respectively, had the most variable sequences in TIBOV; their best base identities between strains were only approximately 50% and 66%, respectively (**Table 1**, **Supplementary Table 4**). In addition, the nucleotide

sequences of VP3, VP7, NS2, and NS3 of strain YNV/KM-1 differed from those of the published strains (**Supplementary Table 4**).

The novel strains were confirmed as TIBOV from a phylogenetic tree of VP1-containing strains belonging to 21 orbiviruses (i.e., RNA-dependent RNA polymerase, RdRP) (**Fig. 2A**, **Supplementary Tables 1** and **2**). The phylogenetic tree of VP2 suggested that existing TIBOV

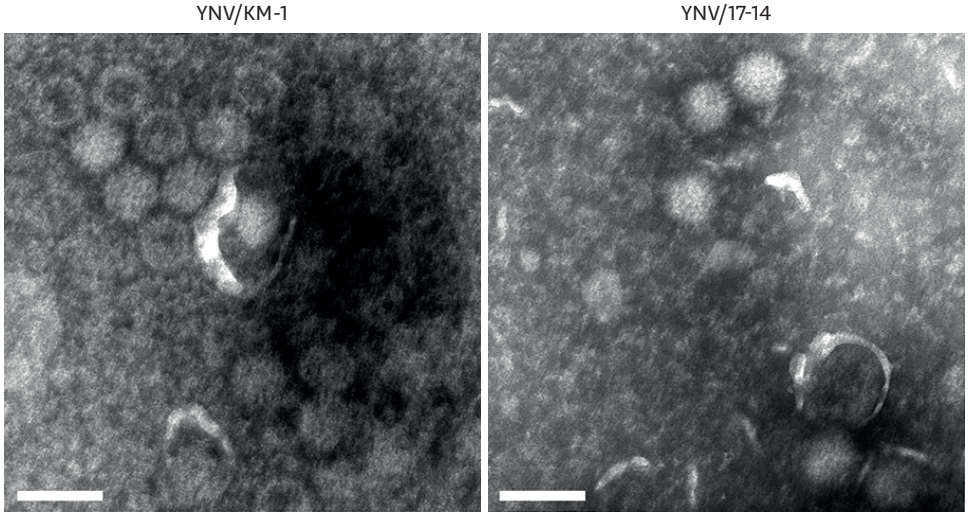

Fig. 1. TEM images of the two viral strains. The virions of strains YNV/KM-1 and YNV/17-14 were observed by TEM (scale bar = 100 nm).
TEM, transmission electron microscopy.

Table 1. Major information of 1–10 segments of the TIBOV strains YNV/KM-1 and YNV/17-14

Segments	Encoded proteins	Data of segments and genes				Nearest TIBOV strains[a]	
		Length (bp)	CDS range	Amino acids (aa)	GenBank No.	Identity (%)	Strains
YNV/KM-1							
Seg1	VP1	3,950	12–3,926	1,304	ON211609	96.99	D181/2008
Seg2	VP2	2,818	14–2,782	922	ON211610	50.86	YNV/17-14
Seg3	VP3	2,769	18–2,717	899	ON211611	82.19	YN15-283-01
Seg4	VP4	1,978	9–1,940	643	ON211612	96.38	DH13C120
Seg5	NS1	1,774	32–1,696	554	ON211613	99.70	YNV/17-14
Seg6	VP5	1,639	27–1,610	527	ON211614	66.29	YNV/17-14
Seg7	VP7	1,165	18–1,067	349	ON211615	79.52	D181/2008
Seg8	NS2	1,142	21–1,100	359	ON211616	84.87	KSB-8/C/09
Seg9	VP6	1,106	15–1,061	348	ON211617	97.13	YN15-283-01
Seg10	NS3	832	22–717	231	ON211618	95.69	YNV/17-14
YNV/17-14							
Seg1	VP1	3,950	12–3,926	1,304	ON211599	100.00	DH13C120
Seg2	VP2	2,883	14–2,845	943	ON211600	50.86	YNV/KM-1
Seg3	VP3	2,769	18–2,717	899	ON211601	99.81	DH13C120
Seg4	VP4	1,978	9–1,940	643	ON211602	99.12	D181/2008
Seg5	NS1	1,774	32–1,696	554	ON211603	99.70	YNV/KM-1
Seg6	VP5	1,637	29–1,609	526	ON211604	66.29	YNV/KM-1
Seg7	VP7	1,165	18–1,067	349	ON211605	98.76	DH13C120
Seg8	NS2	1,142	21–1,100	359	ON211606	96.79	**D181/2008**[b]
Seg9	VP6	1,103	15–1,058	347	ON211607	100.00	YN15-283-01
Seg10	NS3	832	22–717	231	ON211608	95.69	YNV/KM-1

TIBOV, Tibet orbivirus; CDS, complete coding sequences.
[a]The relationships were calculated by the identical bases between complete CDS sequences.
[b]DH13C120, with a nucleic acid identity of 99.26% was closer to YNV/17-14, but segment 8 of DH13C120 has a stop codon within the CDS. Therefore, Seg8 of DH13C120 was removed.

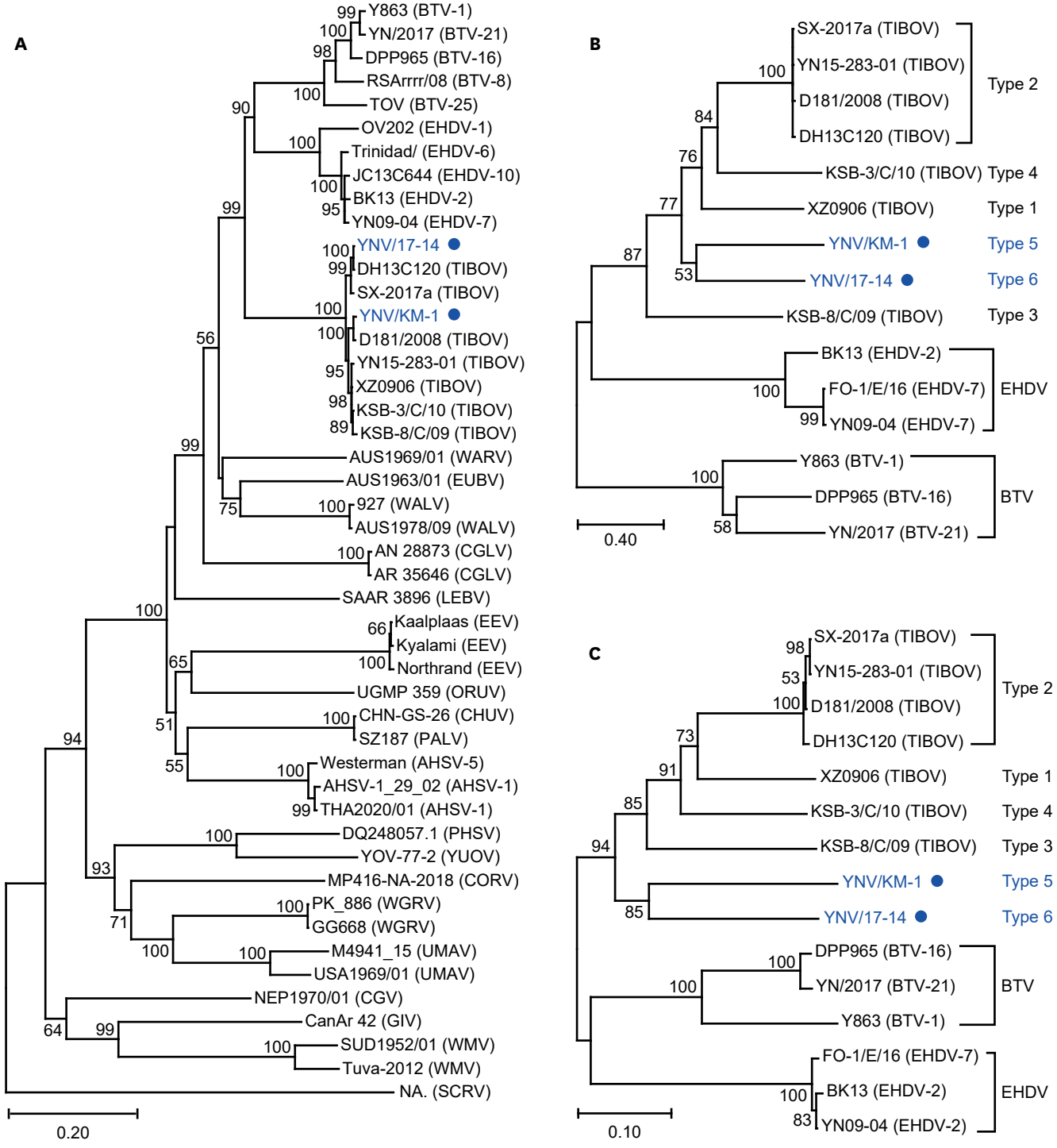

Fig. 2. Phylogenetic trees of VP1, VP2, and VP5. The trees were constructed using the NJ algorithm following the alignments of complete CDS. The two novel strains reported in this study were marked by cycles. Bootstrap values less than 50% were omitted. (A) NJ tree of VP1. Forty-seven strains belonging to 21 *Orbivirus* species were used. (B) NJ tree of VP2. (C) NJ tree of VP5. Putative serotypes of TIBOV are labelled in (B) and (C).
NJ, Neighbor-Joining; CDS, complete coding sequences.

strains were temporarily classified into six putative serotypes (**Fig. 2B**). Such classification was supported by the NJ tree of VP5 (**Fig. 2C**). The VP2 genetic distances between the putative serotypes were more than 0.9, while such values between the type 2 strains were

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less than 0.03 (**Supplementary Table 5**). In the NJ tree of VP7, strain YNV/KM-1 was distinct from the other TIBOV strains (**Supplementary Fig. 1**). In phylogenetic trees of NS1, VP3, VP4, and VP6, the strains were clustered, but the genetic distances between strains were very short (**Supplementary Fig. 2**). Strain YNV/KM-1 was close to YNV/17-14 in VP2 and VP5 (**Fig. 2B and C**) but was close to Japanese strain KSB-8/C/09 in NS2 and NS3 (**Supplementary Fig. 2D and F**).

DISCUSSION

Strains YNV/KM-1 and YNV/17-14 were confirmed as TIBOV by phylogenetic analysis, and the shapes and diameters of these virions were in accordance with the TIBOV reported previously [5].

The VP2 of BTV and its homogenous proteins (e.g., TIBOV VP2) from other orbiviruses were used to determine serotypes of orbiviruses [9,12]. According to the classification by Suda et al. [9], the prototype of TIBOV (XZ0906) [4] was classified as serotype 1. The other four Chinese strains [6-8] were placed in serotype 2, while the two Japanese strains [9] were placed in serotypes 3 and 4, respectively. Therefore, YNV/KM-1 isolated from *Culicoides* spp. in 2019 and YNV/17-14 isolated from *Culicoides jacobsoni* in 2020 [10] were placed respectively in serotypes 5 and 6 in this study (**Fig. 2B**).

Generally, orbiviruses are clustered phylogenetically according to their arthropod hosts (mosquitoes, midges/sandflies, and ticks) [13]. On the other hand, there was no clear correlation between genetic clusters and host species (mosquito or *Culicoides*) among the TIBOV strains (**Fig. 2**, **Supplementary Figs. 1** and **2**). Hence, TIBOV had a wider profile of arthropod hosts than most other orbiviruses.

SUPPLEMENTARY MATERIALS

Supplementary Table 1
The data and GenBank access numbers of TIBOV strains used in this study

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Supplementary Table 2
The information of the *Orbivirus* species used in this study

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Supplementary Table 3
Additional genome information of the two novel TIBOV serotypes

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Supplementary Table 4
Percent identities of genes between the novel TIBOV strains and the published TIBOV strains

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Supplementary Table 5

VP2 genetic distances between TIBOV strains

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Supplementary Fig. 1

Phylogenetic tree of VP7. NJ tree was constructed using VP7 CDS of 9 TIBOV strains, 3 BTV strains, and 3 EHDV strains. The two novel strains reported in this study were marked by cycles. Bootstrap values less than 50% were omitted.

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Supplementary Fig. 2

Six TIBOV phylogenetic trees. The TIBOV NJ trees of VP3 (A), VP4 (B), NS1 (C), NS2 (D), VP6 (E), and NS3 (F) were constructed, respectively. Novel strains reported in this study were marked by cycles and highlighted by blue and red colors, respectively. Bootstrap values less than 50% were omitted.

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REFERENCES

1. International Committee on Taxonomy of Viruses (ICTV). ICTV Master Species List 2021.v1 [Internet]. [place unknown]: ICTV; https://ictv.global/msl. Updated 2021. Accessed 2022 Feb 21.
2. Roy P. Orbiviruses. In: Fields BN, Knipe DM, Howley PM, editors. Fields Virology. 5th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2007, 2541-2568.
3. King S, Rajko-Nenow P, Ashby M, Frost L, Carpenter S, Batten C. Outbreak of African horse sickness in Thailand, 2020. Transbound Emerg Dis. 2020;67:1764-1767.
PUBMED | CROSSREF
4. Li M, Zheng Y, Zhao G, Fu S, Wang D, Wang Z, et al. Tibet orbivirus, a novel Orbivirus species isolated from *Anopheles maculatus* mosquitoes in Tibet, China. PLoS One. 2014;9(2):e88738.
PUBMED | CROSSREF
5. Lei W, Guo X, Fu S, Feng Y, Nie K, Song J, et al. Isolation of Tibet orbivirus, TIBOV, from *Culicoides* collected in Yunnan, China. PLoS One. 2015;10(8):e0136257.
PUBMED | CROSSREF
6. Wang J, Li H, He Y, Zhou Y, Xin A, Liao D, et al. Isolation of Tibet orbivirus from *Culicoides* and associated infections in livestock in Yunnan, China. Virol J. 2017;14(1):105.
PUBMED | CROSSREF
7. Wu D. *Fengkai orbivirus*, a new genotype of Tibet orbivirus isolated from pooled *Culex fatigan* mosquitoes in 2008, in Guangdong, China. In: Workshop for Control of EID. Guangzhou: Emerging Infectious Diseases: 2015.
8. Xing S, Guo X, Zhang X, Zhao Q, Li L, Zuo S, et al. A novel mosquito-borne reassortant oirbivirus isolated from Xishuangbanna, China. Virol Sin. 2017;32(2):159-162.
PUBMED | CROSSREF
9. Suda Y, Murota K, Shirafuji H, Yanase T. Genomic analysis of putative novel serotypes of Tibet orbivirus isolated in Japan. Arch Virol. 2021;166(4):1151-1156.
PUBMED | CROSSREF
10. Duan YL, Yang ZX, Bellis G, Li L. Isolation of Tibet orbivirus from *Culicoides jacobsoni* (Diptera, Ceratopogonidae) in China. Parasit Vectors. 2021;14(1):432.
PUBMED | CROSSREF
11. Maan S, Rao S, Maan NS, Anthony SJ, Attoui H, Samuel AR, et al. Rapid cDNA synthesis and sequencing techniques for the genetic study of bluetongue and other dsRNA viruses. J Virol Methods. 2007;143(2):132-139.
PUBMED | CROSSREF

12. Pritchard LI, Gould AR. Phylogenetic comparison of the serotype-specific VP2 protein of bluetongue and related orbiviruses. Virus Res. 1995;39(2-3):207-220.
PUBMED | CROSSREF

13. Mohd Jaafar F, Belhouchet M, Belaganahalli M, Tesh RB, Mertens PP, Attoui H. Full-genome characterisation of Orungo, Lebombo and Changuinola viruses provides evidence for co-evolution of orbiviruses with their arthropod vectors. PLoS One. 2014;9(1):e86392.
PUBMED | CROSSREF