Polish Journal of Veterinary Sciences Vol. 28, No. 2 (2025), 325–330

DOI 10.24425/pjvs.2025.154950

Short communication

First report on the genomic characterization of Teschovirus B3 in Jiangxi Province, China

W. Liu[1*], D. Zhao[2*], Y. Peng[3], Z. Zhou[4], X. Tang[5], T. Yang[2]

[1] Changde Center for Animal Disease Prevention and Control, Changde, Hunan, China
[2] Laboratory of Animal Pathogenic Microbiology, Yichun University, Yichun, Jiangxi, China
[3] Longshan Center for Animal Disease Prevention and Control, Xiangxi, Hunan, China
[4] Longhui Center for Animal Disease Prevention and Control, Shaoyang, Hunan, China
[5] Hunan Center for Animal Disease Prevention and Control, Changsha, Hunan, China

Correspondence to: Taotao Yang, e-mail: 1078249169@qq.com; Xiaoming Tang, e-mail: 28860002@qq.com
* These authors contributed equally to this article.

Abstract

The genus *Teschovirus* consists of two species, *Teschovirus A* and *Teschovirus B*, with over 19 genotypes. This study sequenced the near-complete genomes of the PTV YC2 strain, previously isolated. Comparative analyses revealed nucleotide and amino acid homologies between PTV-YC2 and other PTV strains ranging from 69.7% to 93.2% and 75.6% to 99.0%, respectively. Genetic divergence analysis of the P1, polyprotein and 2C-3CD genes confirmed that PTV-YC2 belonged to the *Teschovirus B* species. Phylogenetic analyses showed a close evolutionary relationship between YC2 and the HuN42 strain, the prototype of the TV-B3 genotype, leading to PTV-YC2's categorization as TV-B3. This study is the first to document the prevalence of the TV-B3 genotype in Jiangxi Province, China.

Keywords: *Porcine teschovirus*, *Teschovirus B*, genotype, species

Introduction

Porcine teschovirus (PTV) is a single positive-strand RNA virus, approximately 7.1 kb in size, with an open reading frame encoding polyproteins cleaved into four structural proteins (1A-D) and eight Non-structural proteins (L, 2A-C and 3A-D) are cleaved (Zell et al. 2001). Structural protein genes are highly variable and always used for genotyping, while non-structural protein genes are relatively conserved and help differentiate PTV species (Yang et al. 2020a, Yang et al. 2020b).

PTV contains two species, *Teschovirus A* and *Teschovirus B*, with the genus *Teschovirus* of the *Picornaviridae* family. The 2019 ICTV report states that *Teschovirus A* has 14 genotypes (PTV-1-14) (ICTV 2022) and identifies four isolates representing three new genotypes (TV-B1-3) as the newly created species *Teschovirus B* (Yang et al. 2018a, Oba et al. 2018).

Two additional genotypes from interspecies recombinants have also been reported (Oba et al. 2018, Yang et al. 2020a). Although PTV prevalence is well documented in China, *Teschovirus B* strains are rare (Sun et al. 2015, Yang et al. 2018a, Yang et al. 2018b). Here, we report the PTV-YC2 isolate, which first confirms the existence of the TV-B3 genotype in Jiangxi, China.

Table1. Homology comparison between the PTV YC2 isolate and previously reported PTV genotype strains.

Genotypes	% nucleotide and deduced amino acid identities of polyprotein:	
	nt[a]	aa[b]
PTV 1	70.2-71.0	77.0-77.3
PTV 2	70.6-71.3	77.3-77.8
PTV 3	70.4-70.9	77.0-77.3
PTV 4	70.3-70.7	76.7-77.5
PTV 5	70.3-71.0	76.9-77.3
PTV 6	70.0-70.8	76.4-76.9
PTV 7	70.5	77.2
PTV 8	70.1-70.7	75.9-77.3
PTV 9	70.2-70.4	76.9-77.1
PTV 10	69.7	76.9
PTV 11	70.2-70.4	76.9-77.3
PTV 12	70.1-70.6	75.9-76.8
PTV 13	69.8	75.6
PTV 15	79.2	84.9
PTV 16	81.0	85.4
PTV 17	70.5	76.5
PTV 18	70.2	76.6
PTV 19	70.1	76.4
Teschovirus B1	83.4-84.6	92.0-92.4
Teschovirus B2	83.9	92.5
Teschovirus B3	93.2	99.0

[a] Nucleotide sequence identity.
[b] Deduced amino acid sequence identity.

Materials and Methods

YC2 was isolated from the stool sample of an infected pig in western Jiangxi, China in our previous work (Yang et al. 2023). To obtain the complete genome sequence of YC2, the viral stocks of the YC2 strain propagated in ST cell monolayers were used for RNA extraction (Vazyme, Nanjing, China) and cDNA synthesis (Thermo Scientific, Waltham, MA, USA). First, the VP1 and 3ABC genes were amplified and sequenced using primers designed in our previous study (Yang et al. 2018a). Subsequently, the primers designed based on the obtained gene sequences and the conserved regions of the 5′ and 3′ UTRs were used to amplify the remaining blanking regions. The PCR-amplified program was referred to as previously described (Yang et al. 2018a). Finally, the PCR products were purified using agarose gel electrophoresis and sequenced (Sangon, Shanghai, China).

The sequencing results were spliced with the SeqMan program using DNAStar software (Burland 2000). Multiple sequence alignment between YC2 and PTV reference genotype strains retrieved from GenBank was performed using Mafft software (Katoh and Toh 2010). The YC2 sequence was then edited using the EditSeq program in DNAStar software (Burland 2000). Homology analysis and genetic divergence between PTV genotype strains were calculated with the MegAlign program using DNAStar software and the p-distance method using MEGA 6.06 software, respectively (Burland 2000, Tamura et al. 2013). Phylogenetic analyses for amino acid (aa) sequences were performed using the maximum likelihood (ML) method in MEGA 6.06 (Tamura et al. 2013). The Jones--Taylor-Thornton (JTT) model with the proportion of invariant sites and gamma-distributed rate heterogeneity (G+I) was used to construct the ML tree, with 1000 bootstrap replications to support the phylogeny.

Results and Discussion

Unfortunately, the amplification of genes at the 5’ UTR beginning and 3’ UTR end was unsuccessful. Ultimately, a nearly complete genome of 6800 nucleo-

Table 2. Estimates of evolutionary divergence between PTV YC2 and the other PTV genotypes of *Teschovirus* based on the aa sequences of the P1, ORF and 2C-3CD genes.

Species	Genotypes	Genes		
		P1	ORF	2C-3CD
Teschovirus A	PTV-1	0.351±0.015	0.223±0.008	0.136±0.010
	PTV-2	0.342±0.015	0.220±0.009	0.138±0.010
	PTV-3	0.352±0.016	0.223±0.009	0.133±0.010
	PTV-4	0.350±0.015	0.225±0.008	0.141±0.010
	PTV-5	0.348±0.015	0.223±0.009	0.139±0.010
	PTV-6	0.352±0.015	0.227±0.008	0.140±0.010
	PTV-7	0.357±0.015	0.223±0.009	0.135±0.010
	PTV-8	0.361±0.015	0.227±0.008	0.140±0.010
	PTV-9	0.355±0.015	0.225±0.009	0.139±0.010
	PTV-10	0.347±0.015	0.228±0.009	0.142±0.011
	PTV-11	0.348±0.015	0.223±0.009	0.140±0.010
	PTV-12	0.360±0.016	0.228±0.009	0.139±0.010
	PTV-13	0.367±0.016	0.239±0.009	0.149±0.011
	PTV-14	0.349±0.016	NA[a]	NA
	PTV-17	0.359±0.016	0.229±0.009	0.144±0.010
	PTV-18	0.364±0.015	0.229±0.009	0.139±0.011
	PTV-19	0.360±0.015	0.231±0.009	0.144±0.011
Interspecies recombinants	PTV-15	0.348±0.015	0.148±0.008	0.010±0.003
	PTV-16	0.333±0.015	0.139±0.007	0.006±0.002
Teschovirus B	TV-B1	0.156±0.011	0.074±0.005	0.016±0.004
	TV-B2	0.147±0.012	0.071±0.006	0.015±0.004
	TV-B3	0.007±0.003	0.01±0.002	0.005±0.002

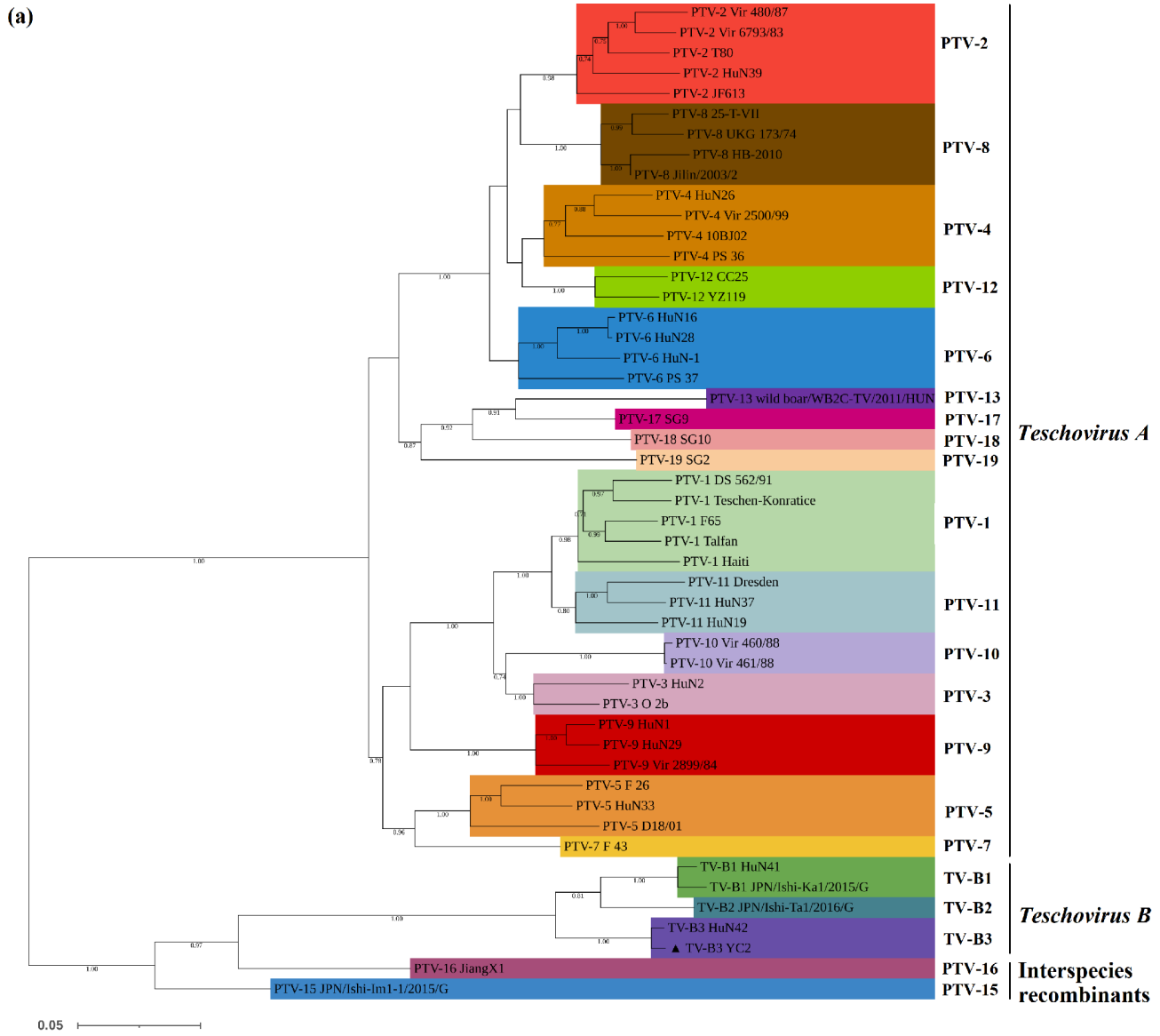
[a] Not applicable, genome sequence of PTV 14 was incomplete.

tides containing the complete polyprotein coding sequence was obtained and then submitted to GenBank (accession numbers PP839288). Thus, the YC2 isolate contains a polyprotein-encoding genome of 6,645 nucleotides that encodes a polyprotein of 2,215 amino acids. Identity analyses of the polyprotein coding genome sequence of the YC2 strain with those of the PTV genotype reference strains revealed the highest homology to the TV-B3 strain with nucleotide and deduced aa sequence identities of 93.2% and 99.0%, respectively (Table 1).

To assess the taxonomy of the PTV-YC2 isolate, we calculated genetic divergences of the P1, polyprotein, and 2C-3CD genes between YC2 and known PTV genotypes (PTV-1–19 and TV-B1–3). The average genetic distances for these genes between YC2 and *Teschovirus A* genotypes ranged from 0.342 to 0.367 (P1), 0.220 to 0.239 (polyprotein), and 0.133 to 0.149 (2C-3CD). For interspecies recombinant genotypes (PTV-15 and PTV-16), the distances were 0.333 to 0.348 (P1), 0.139 to 0.148 (polyprotein), and 0.006 to 0.010 (2C-3CD). In comparison, distances to *Teschovirus B* genotypes (TV-B1–B3) ranged from 0.007 to 0.156 (P1), 0.01 to 0.074 (polyprotein), and 0.005 to 0.016 (2C-3CD) (Table 2). According to ICTV species delimitation criteria (ICTV 2022), YC2 is classified as *Teschovirus B*.

To further characterize the evolutionary relationship of the obtained PTV-YC2 isolate, phylogenetic analyses based on the polyprotein, P1 and 3CD gene a sequences were performed. In the three phylogenetic trees based on the polyprotein, P1 and 3CD genes, the YC2 isolate was found to fall into the *Teschovirus B* group, which also includes Chinese (HuN41 and HuN42) and Japanese (JPN/Ishi-Ka1/2015/G and JPN/Ishi-Ta1/2016/G) strains (Fig 1a-c). These results also suggest that YC2 belongs to the *Teschovirus B* species. Furthermore, phylogenetic trees of polyprotein and P1 genes showed that YC2, clustered in a monophyletic branch, represents the new genotype of TV-B3 with bootstrap values of 100%, along with the closely related and unique HuN42 strain, which was isolated from Hunan Province (Fig 1a, and b). Therefore, YC2 was verified as genotype TV-B3.

PTV infects domestic pigs and wild boars, including only one species of virus, *Teschovirus A*, before 2018 (Zell et al. 2017). By 2018, our previous study

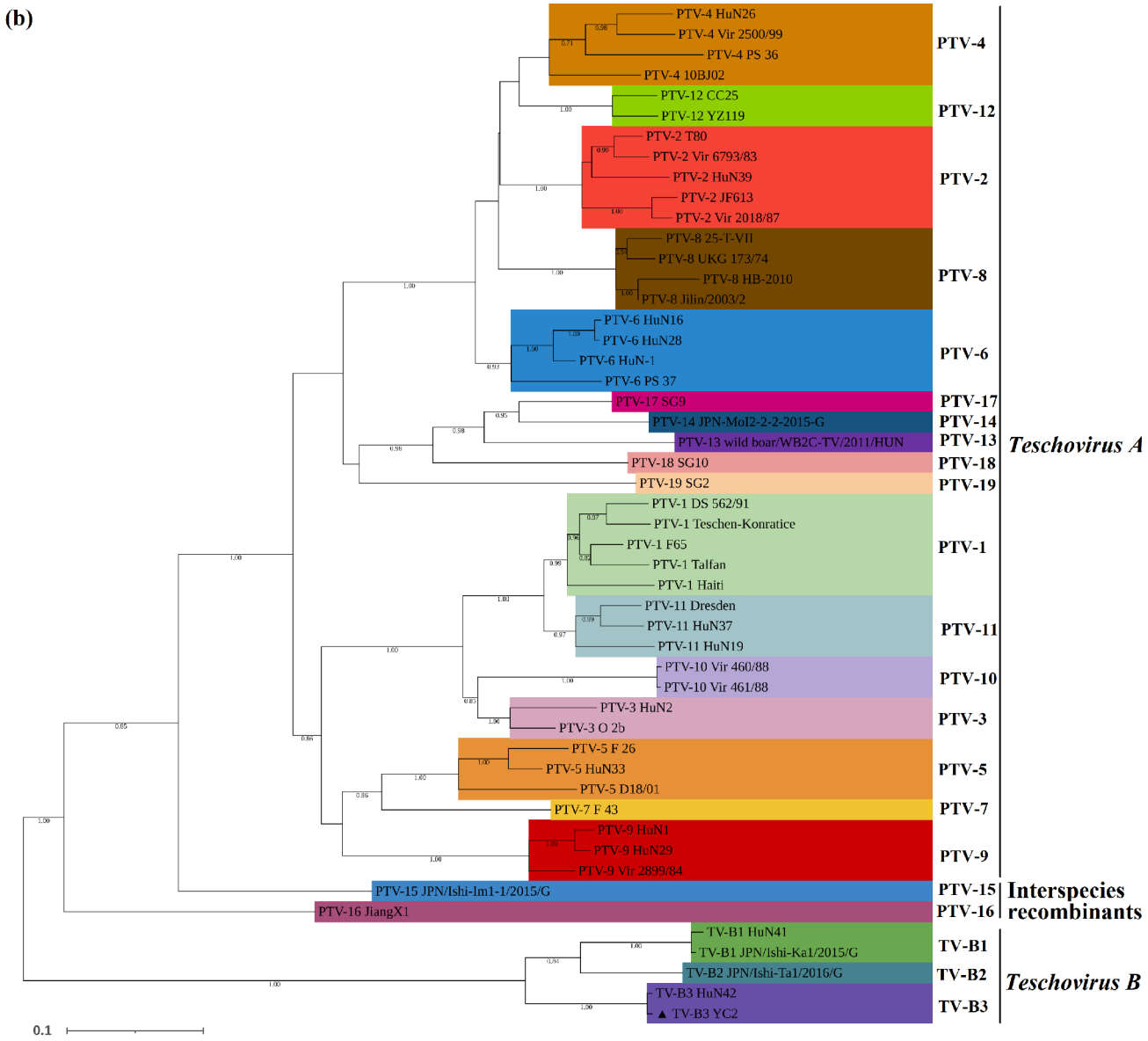


discovered for the first time two PTV strains that were similar to the previously reported PTVs but had a large genetic distance, suggesting that there may be a new PTV virus species in the *Teschovirus* genus (Yang et al. 2018a). In the same year, Oba et al. identified similar PTV strains in swine fecal samples using the metagenomic sequencing method (Oba et al. 2018).

In 2019, the *Picornaviridae* study group proposed the creation of a new species, *Teschovirus B*, in the genus *Teschovirus* and listed the species delimitation criteria: members of the same species in the genus *Teschovirus* exhibit less than 20%, 30%, and 10% divergence in the sequences of the polyprotein, P1, and 2C+3CD genes, respectively, and share a common genome organization and natural host range (ICTV 2022). Thus, only four circulating *Teschovirus B* strains have been reported worldwide based on these criteria (Yang et al. 2018a, Oba et al. 2018). Therefore, the newly discovered YC2 isolate in this study represents the fifth *Teschovirus B* strain and is the second strain of the TV-B3 genotype reported globally.

PTV contained only 11 genotypes before the 21st century (Zell et al. 2001). With the development of molecular biology technology, especially the application of the metagenomic sequencing method in virus identification and discovery, a large number of new PTV genotypes have been discovered in recent years (Boros et al. 2012, Cano-Gomez et al. 2017, Yang et al. 2018a, Oba et al. 2018, Yang et al. 2020a, Yang et al. 2020b, Yang et al. 2023). These newly discovered genotypes, particularly present in *Teschovirus B*, have significantly enriched the understanding of PTV. In the meantime, there may still be many undefined new PTV strains that represent potential new genotypes (Yang et al. 2018a, Yang et al. 2020b). The classification of PTV still needs further research and discovery.

In conclusion, a near complete genome sequence of the YC2 isolate was completed in this study. Through



genetic evolutionary analysis, YC2 was identified as a member of the TV-B3 genotype in *Teschovirus B*. This was the first time that the prevalence of the TV-B3 genotype strain was reported in Jiangxi Province, China.

Acknowledgements

This work was supported by the Natural Science Foundation of Jiangxi Province (20212BAB215032).

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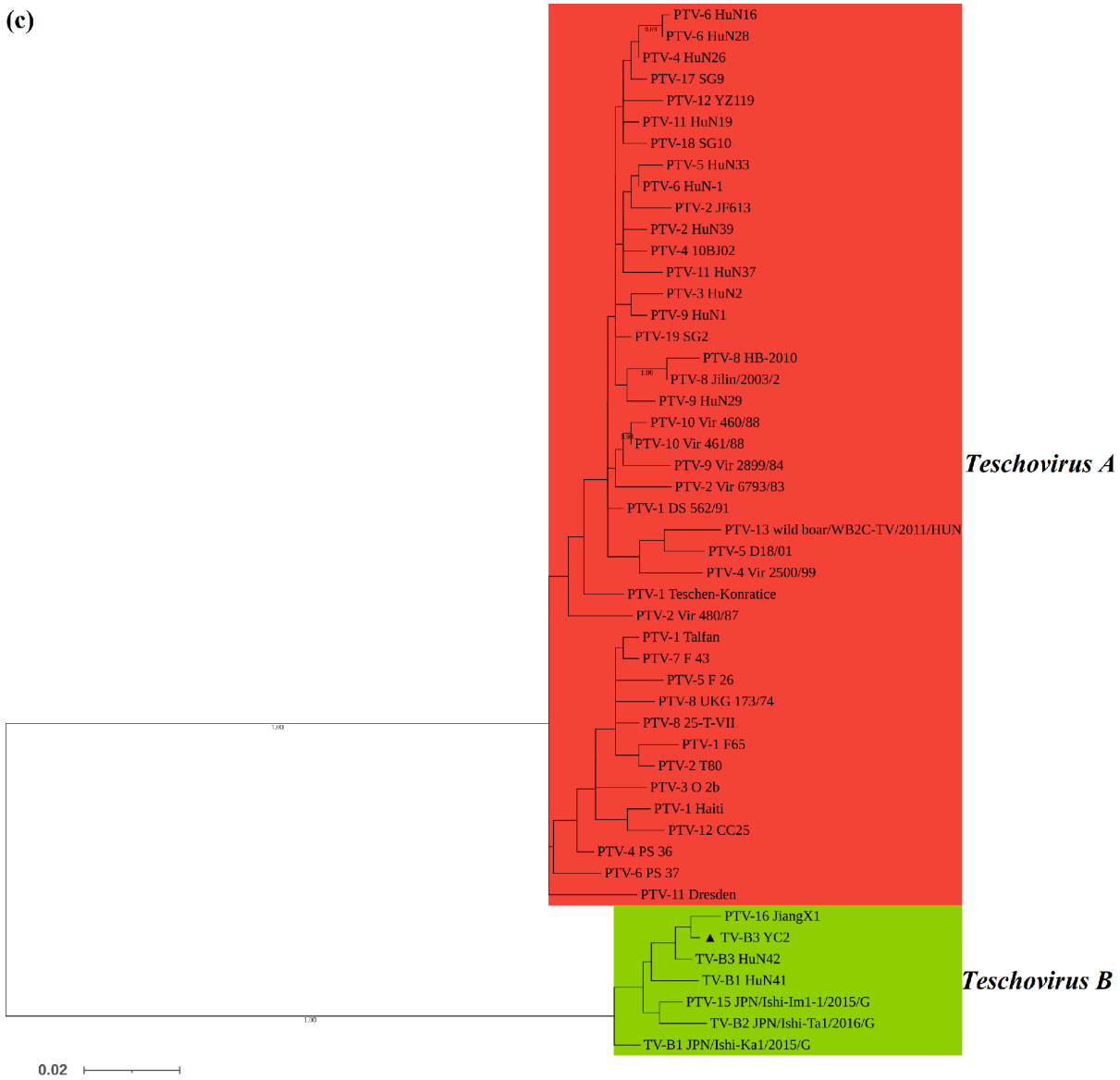

Fig. 1. Evolutionary relationship of the PTV-YC2 isolate. Phylogenetic trees based on the gene aa sequences of polyprotein (a), P1 (b) and 3CD (c) were constructed using the maximum likelihood method based on the Jones-Taylor-Thornton (JTT) model with gamma-distributed rates and proportions of invariant sites (G+I) created using MEGA 6.06 software. A phylogeny test was performed on all trees using 1,000 bootstrap replicates, and only values >70% of the trees where the associated taxa were grouped are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The phylogenetic trees were color annotated using iTOL version 6 (https://itol.embl.de) (Letunic and Bork 2024). The YC2 isolate identified in the present study is indicated by a triangle.

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