Epidemiology and genetic characteristics of pigeon circovirus (PiCV) in eastern China

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Abstract Pigeon circovirus (PiCV) is one of four viruses in the family *Circoviridae* that affect young pigeons around the world. We collected 158 serum or tissue samples from six poultry farms in eastern China to investigate the prevalence and genetic characteristics of PiCV in Chinese pigeons. We tested for PiCV using a PCR assay and found that PiCV was present in 80.7 % (88/109) of diseased pigeons and 63.3 % (31/49) of healthy pigeons; overall, 75.3 % (119/158) of samples were PiCV positive. One PiCV-positive sample from each poultry farm was randomly chosen for amplification of the complete PiCV genome by inverse primer PCR (IP-PCR). The six genomic PiCV strains were designated as AHBZ (KJ704801), HBLF-E2 (KJ704802), JSJN (KJ704803), NJPK-21 (KJ704804), SDDZ (KJ704805) and SHWH-AB4 (KJ704806). We compared these new PiCV genomes to six publicly available PiCV genomes and found that the *Rep* and *Cap* genes had sequence identity ranging from 93.8 % to 100 % and 79.1 % to 100 %, respectively. In a phylogenetic analysis, PiCV and eight other members of the genus *Circovirus* were sister to chicken anemia virus (CAV), the only member of genus *Gyrovirus*. The results of this study provide evidence that PiCV is present in Chinese pigeons at a high rate and that PiCV is a viral lineage that is distinct from CAV.

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Introduction

Pigeon circovirus (PiCV), also known as columbid circovirus (CoCV), is a small, circular, non-enveloped, single-stranded DNA virus that belongs to the genus *Circovirus* of the family *Circoviridae* [1]. PiCV was first recognized in the USA in 1993 [2], and it was later reported in Australia [3] and several European countries, including Northern Ireland [4], England [5], Germany [6], France [7], Italy [8] and Belgium [9]. PiCV infection has been identified in other parts of the world in recent years [10], but little is known about the pathogenesis of the virus. It was reported recently that PiCV is responsible for a complex and variable immune suppression syndrome that mostly affects young pigeons, called "young pigeon disease syndrome" (YPDS) [11]. PiCV infection is generally characterized by a wide range of clinical symptoms, including lethargy, anorexia, weight loss, respiratory diseases, diarrhoea, and other non-characteristic behaviours [12, 13].

The complete genome of PiCV was first cloned from pigeon tissues in 2000 [14]. The average PiCV genome size is 2.0 kb, with minor length variations among different isolates [15]. Viral genomes are characterized by two major open reading frames (ORF), namely ORF V1, composed of 318 amino acids (nt 41–994), encoding the replication-associated protein (*Rep*), and ORF C1 in the opposite orientation, composed of 274 amino acids (nt 1165–1988) [16], encoding the only structural protein of the virion—capsid protein (*Cap*) [17]. An additional ORF, ORF C2, is

composed of 250 amino acids (nt 411–791), encoding a hypothetical protein [17, 18]. The functions of the other ORFs are unknown.

The family *Circoviridae* includes two genera: *Gyrovirus* and *Circovirus* [19]. Presently, the chicken anemia virus (CAV) is the only member of the genus *Gyrovirus*, while the genus *Circovirus* includes several members, including pigeon circovirus (PiCV), porcine circovirus (PCV), psittacine beak and feather disease virus (BFDV) and canary circovirus (CaCV) [20, 21]. Thus far, there are no available culture methods for PiCV, but a PCR assay based on specific PiCV DNA sequences can be used to detect the presence of the virus [22]. In this study, we detected PiCV in Chinese pigeons by PCR and amplified complete PiCV genomes by inverse primer PCR (IP-PCR). We used bioinformatics software to align and analyse the complete PiCV genome sequences from the samples collected in this study as well as those of nine other members of the family *Circoviridae* available in the GenBank database. We also estimated a phylogeny of PiCV based on analysis of *Rep* and *Cap* genes.

Materials and methods

Sample sources

A total of 109 infected pigeons displaying clinical symptoms (such as respiratory signs, weight loss, and diarrhea) and 49 healthy pigeons were collected from poultry farms in the six provinces (Shandong, Anhui, Zhejiang, Shanghai, Hebei and Jiangsu) with the highest pigeon population densities in eastern China (Table 1). Serum or tissue samples were collected from pigeons and immediately frozen at −80 °C.

Reagents

DNA and gel extraction kits were purchased from Geneaid (USA). DL2000 DNA Marker, Pfu DNA polymerase, Taq DNA polymerase, 10× buffer, dNTP mixture and a pMD18-T vector kit were purchased from TaKaRa (Dalian, China). DH5α competent cells were purchased from Tiangen (Beijing, China). All reagents were high-purity and intended for analytical use.

Primer design and synthesis

Two pairs of primers, one for PiCV detection (PiCV-1/2) and the other for amplification of complete genomes (PiCV-JST1/2), were designed according to the published genome sequence of PiCV available in the GenBank database (Table 2). Primers were synthesized by Invitrogen (Shanghai, China).

PCR detection of PiCV

Genomic DNA was extracted from 200 μl of serum (10 %, w/v) or from approximately 25 mg of tissue samples using a commercial DNA extraction kit according to the manufacturer's instructions. The extracted DNA was eluted twice with 25 μl of sterile distilled water to give a final extract volume of 50 μl.

The genomic DNA was used as template to perform PCR for the detection of PiCV. PCR reaction mixtures (25 μL) contained 18 μL of ddH$_2$O, 2.5 μL of 10× buffer, 1 μL each of primer pairs PiCV-1 and PiCV-2 (10 μM each), 2 μL of DNA template, and 0.5 μL of Taq DNA polymerase (5 U/μL). PCR was initiated by heating for 5 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 60 °C, 40 s at 72 °C and a final extension for 10 min at 72 °C. The amplification products (2 μL) were analyzed by electrophoresis in a 2 % TAE agarose gel and visualized under ultraviolet light.

Cloning and sequencing of the PiCV genome

Complete PiCV genomes were amplified by IP-PCR using the inverse primers PiCV-JST1 and PiCV-JST2. Templates were randomly chosen PiCV-positive DNA samples from six poultry farms (one from each geographic region). IP-PCR reaction mixtures (50 μL) contained 21 μL of Pfu DNA polymerase, 4 μL of DNA template, 2 μL each of the primers, and 21 μL of ddH$_2$O. The PCR protocol was the same as described above. PCR amplification products with the expected sizes were purified using a commercial gel extraction kit according to the manufacturer's instructions.

The recovered DNA was cloned into the pMD18-T vector and used to transform DH5α competent cells using the heat shock method. The positive clones were confirmed by PCR and Sanger sequencing.

Table 1 Pigeon serum and tissue samples used for PiCV detection

Region	Samples from diseased pigeons[a]	Samples from healthy pigeons	Total
Zhenjiang	17	6	23
Jiangsu	25	12	37
Anhui	22	8	30
Shandong	14	5	19
Shanghai	16	7	23
Hebei	15	11	21
Total	109	49	158

[a] Pigeons displayed clinical signs of illness, such as respiratory distress, weight loss, and diarrhea

Table 2 Primer sequences used in this study

Primer	Nucleotide sequence	Expected amplicon length
PiCV-1	5′-TTGAAAGGTTTTCAGCCTGGC-3′	325 bp
PiCV-2	5′-AGGAGACGAAGGACACGCCTC-3′	
PiCV-JST1	5′-CCCCACTGAAGAAGAGATTAAGAGCCTGGAAACGTG-3′	2040 bp
PiCV-JST2	5′-CTTAATCTCTTCTTCAGTGGGGTTGTTCAA-3′	

Table 3 PiCV infection results

Region	Samples from diseased pigeons[a]	Samples from healthy pigeons	Total
Zhenjiang	14 (14/17, 82.4 %)	3 (3/6, 50.0 %)	17 (17/23, 73.9 %)
Jiangsu	21 (21/25, 84.0 %)	7 (7/12, 58.3 %)	28 (28/37, 75.7 %)
Anhui	17 (17/22, 77.3 %)	5 (5/8, 62.5 %)	22 (22/30, 73.3 %)
Shandong	11 (11/14, 78.6 %)	3 (3/5, 60.0 %)	14 (14/19, 73.7 %)
Shanghai	13 (13/16, 81.3 %)	5 (5/7, 71.4 %)	18 (18/23, 78.3 %)
Hebei	12 (12/15, 80.0 %)	8 (8/11, 72.7 %)	20 (20/21, 95.2 %)
Total	88 (88/109, 80.7 %)	31 (31/49, 63.3 %)	119 (119/158, 75.3 %)

[a] Pigeons displayed clinical signs of illness, such as respiratory distress, weight loss, and diarrhea

Sequence alignment and analysis

Sequences were first identified by BLAST search against the NCBI database. DNASTAR 7.1 and ClustalX 1.8 software were used to perform alignments and sequence comparisons [23]. Phylogenies were inferred from distance matrices using the neighbor-joining method with a boot-strap test of 1000 iterations in MEGA5 [24].

Results

PCR detection of PiCV infection

An approximately 338-bp specific fragment was amplified. The specific PCR products were identified as PiCV by sequencing and BLAST analysis. Of the 158 samples, 119 were PiCV positive (positive rate, 75.3 %). Among the samples, 88 samples from the 109 unhealthy pigeons (80.7 %) and 31 samples from the 49 healthy pigeons (63.3 %) were PiCV positive (Table 3). PiCV was detected in samples from both diseased and healthy pigeons.

Amplification of PiCV genomes

An approximately 2040-bp complete genome fragment was obtained from PiCV-positive samples from six poultry farms. BLAST alignment results confirmed that six of the recovered genomes were PiCV, and they were designated as the AHBZ, HBLF-E2, JSJN, NJPK-21, SDDZ and SHWH-AB4 strains. The GenBank IDs of these six PiCV complete genomes were KJ704801, KJ704802, KJ704803, KJ704804, KJ704805, and KJ704806, respectively.

Sequence analysis of PiCV genomes

PiCV is a member of the genus *Circovirus* and the family *Circovirdae*, so we included nine publicly available genomes to represent the diversity of the family *Circovirdae*. The genomes were downloaded from GenBank and included chicken anemia virus (CAV), porcine circovirus (PCV), duck circovirus (DuCV), goose circovirus (GoCV), beak and feather disease virus (BFDV), canary circovirus (CaCV), gull circovirus (GuCV), starling circovirus (StCV), raven circovirus (RaCV), and six PiCV strians (PiCV-zj1, NI7050, Ger1, Bel936, FraA4042, US93A) from China, the USA, and four European countries (Table 4).

Sequence analysis demonstrated that the PiCV genome contained two main ORFs, ORF V1 (ORF 1) and ORF C1 (ORF2). As in other members of the genus *Circovirus*, these genes encode the viral replication-associated protein (*Rep*) and the viral capsid protein (*Cap*), respectively. ORF V1 corresponds to nt 41–994 in the nucleotide sequence and encodes 318 amino acids. ORF C1 corresponds to nt 1165–1988 in the nucleotide sequence and encodes 274 amino acids. Notably, the PiCV coding sequence region of ORF V1 and ORF C1 had a conserved nine-nucleotide sequence (TAGTATTAC; Table 5).

Sequence similarity of PiCV genomes

The 12 complete PiCV genome sequences were analysed to assess sequence similarity. The percent sequence similarity among the six complete PiCV genomes in this study ranged from 88.0 % to 100 %, and from 86.9 % to 100 % in a comparison with six other PiCV genomes (Fig. 1), suggesting that PiCV strains maintain an evolutionary signal

despite their distribution, and there were no obvious geographical differences in these strains.

We also assessed the sequence similarity of *Rep* and *Cap* genes of the 12 complete PiCV genomes. The sequence similarity of the six *Rep* and *Cap* datasets generated in this study ranged from 93.9 % to 100 % and 83.1 % to 98.5 %, respectively. The sequence similarity ranged from 93.8 % to 100 % and 79.1 % to 100 % in a comparison with the six other *Rep* and *Cap* sequences available from GenBank (Fig. 2 and Fig. 3). The PiCV *Cap* sequences were more variable than the relatively conserved *Rep* sequences.

Sequence analysis of circoviruses

The sequence similarity rate of the 11 circovirus genome sequences from GenBank (Table 4) ranged from 30.2 % to 82.7 %, and the sequence similarity between the 11 circoviruses and the 12 PiCV genomes ranged from 31.4 % to 65.0 % (Fig. 4). It was notable that the sequence similarity between the CAV genome and 22 other circovirus genomes ranged from 30.2 % to 35.7 %, indicating that the only member of the genus *Gyrovirus*, CAV, is distantly related to the other circoviruses.

In addition, we constructed a phylogenetic tree based on the 23 complete genomic sequences of the family *Circoviridae* listed in Table 4 (Fig. 5). In the phylogenetic tree, the 23 circovirus genomes formed two distinct groups. The first branch included the 22 genomes of circoviruses from different hosts, suggesting that they had a close genetic relationship. As the only member of the genus *Gyrovirus*, one CAV strain was grouped into the second branch alone.

Discussion

As with other avian circoviruses, PiCV was detected in a variety of tissues and is therefore likely to be present in many secretions [25]. However, there is little information on the prevalence of PiCV infection [26]. We found that PiCV is widely distributed in eastern Chinese pigeons, and there are no obvious differences in the geographical distribution of this virus. The fact that PiCV was detected in both healthy and diseased pigeons implies a potential threat to the pigeon-breeding industry. Histopathological examinations have revealed lymphocyte depletion in the primary and secondary lymphoid tissues in PiCV-infected pigeons, and PiCV infection is thought to cause immune suppression, like other circovirus infections [27]. When the immune system is compromised, secondary infections

Table 4 GenBank sequences used in this study

Strain name	Accession no.	Origin	Host	Length (bp)
PiCV-zj1	DQ090945	China	Pigeon	2039
NI7050	AJ298230	UK	Pigeon	2036
Ger1	AF252610	Germany	Pigeon	2037
Bel936	DQ915956	Belgium	Pigeon	2038
FraA4042	DQ915960	France	Pigeon	2037
US93A	DQ915961	USA	Pigeon	2037
CaCV	AJ301633	Australia	Canary	1952
RaCV	DQ146997	Australia	Raven	1898
StCV	DQ172906	Germany	Starling	2063
GuCV	DQ845074	UK	Gull	2035
BFDV	AF311295	Australia	*Psephotus haematogaster*	2018
DuCV-WF0701	EU022375	China	Duck	1991
DuCV-LY0701	EU022374	China	Duck	1988
GoCV	NC003054	USA	Goose	1821
PCV2	EF394777	Canada	Pig	1767
PCV1	AY754015	Australia	Pig	1759
CAV-CUX-1	M55918	USA	Chicken	2319

Table 5 Characteristics of ORFs from different PiCV strains

Strain name	Host	Length (bp)	ORF V1 (*Rep*)	ORF C1 (*Cap*)	Nonamer sequence
PiCV-zj1	Pigeon	2039	318[a] (nt 41[b])	275[a] (nt 1167[b])	TAGTATTAC
NI7050	Pigeon	2036	318 (nt 41)	274 (nt 1165)	TAGTATTAC
Ger1	Pigeon	2037	318 (nt 41)	274 (nt 1166)	TAGTATTAC
Bel936	Pigeon	2038	318 (nt 41)	274 (nt 1167)	TAGTATTAC
FraA4042	Pigeon	2037	318 (nt 41)	274 (nt 1166)	TAGTATTAC
US93A	Pigeon	2037	318 (nt 41)	274 (nt 1166)	TAGTATTAC
AHBZ	Pigeon	2035	318 (nt 41)	274 (nt 1166)	TAGTATTAC
HBLF-E2	Pigeon	2037	318 (nt 41)	274 (nt 1166)	TAGTATTAC
JSNJ	Pigeon	2039	318 (nt 41)	274 (nt 1166)	TAGTATTAC
NJPK-21	Pigeon	2035	318 (nt 41)	274 (nt 1166)	TAGTATTAC
SDDZ	Pigeon	2037	318 (nt 41)	274 (nt 1166)	TAGTATTAC
SHWH-AB4	Pigeon	2031	318 (nt 41)	274 (nt 1166)	TAGTATTAC

[a] Number of amino acids encoded by ORF

[b] nt position at which ORF starts

Percent Identity

Divergence	1	2	3	4	5	6	7	8	9	10	11	12		
1		88.6	88.0	100.0	97.2	90.9	90.2	97.0	97.8	95.2	88.0	95.0	1	AHBZ China.seq
2	12.4		87.1	88.6	89.3	97.5	86.9	88.8	89.1	88.8	87.1	88.9	2	HBLF-E2 China.seq
3	13.0	14.1		88.0	88.3	89.0	88.9	88.0	88.6	88.4	100.0	88.3	3	JSNJ China.seq
4	0.0	12.4	13.0		97.2	90.9	90.2	97.0	97.8	95.2	88.0	95.0	4	NJPK-21 China.seq
5	2.9	11.6	12.7	2.9		91.5	91.0	96.8	97.7	96.6	88.3	95.5	5	SDDZ China.seq
6	9.8	2.6	11.9	9.8	9.1		88.9	90.9	91.3	90.8	89.0	91.0	6	SHWH-AB4 China.seq
7	10.5	14.4	12.0	10.5	9.6	12.0		90.3	91.0	90.0	88.9	90.4	7	Bel936 Belgium.seq
8	3.1	12.1	13.1	3.1	3.3	9.7	10.4		97.8	95.4	88.0	95.4	8	FraA4042 France.seq
9	2.2	11.8	12.4	2.2	2.3	9.3	9.6	2.2		95.8	88.6	95.5	9	Ger 1 Germany.seq
10	4.9	12.1	12.6	4.9	3.5	9.9	10.6	4.7	4.4		88.4	96.1	10	NI7050 UK.seq
11	13.0	14.1	0.0	13.0	12.7	11.9	12.0	13.1	12.4	12.6		88.3	11	PiCV-zj1 China.seq
12	5.2	12.0	12.7	5.2	4.6	9.6	10.2	4.8	4.7	4.0	12.7		12	US93A USA.seq
	1	2	3	4	5	6	7	8	9	10	11	12		

Fig. 1 Percent identity of 12 PiCV genome sequences based on amino acids. Samples 1–6 are PiCV sequences obtained in this study. Samples 7–12 are PiCV sequences from GenBank

Percent Identity

Divergence	1	2	3	4	5	6	7	8	9	10	11	12		
1		96.8	94.3	100.0	97.9	96.8	95.3	96.4	98.1	95.4	94.3	96.0	1	AHBZ Rep.seq
2	3.3		94.9	96.8	96.9	100.0	95.0	96.6	97.2	95.6	94.9	96.6	2	HBLF-E2 Rep.seq
3	5.9	5.3		94.3	93.9	94.9	94.5	93.8	94.4	93.7	100.0	93.9	3	JSNJ Rep.seq
4	0.0	3.3	5.9		97.9	96.8	95.3	96.4	98.1	95.4	94.3	96.0	4	NJPK-21 Rep.seq
5	2.1	3.2	6.3	2.1		96.9	95.8	96.2	98.3	95.6	93.9	96.0	5	SDDZ Rep.seq
6	3.3	0.0	5.3	3.3	3.2		95.0	96.6	97.2	95.6	94.9	96.6	6	SHWH-AB4 Rep.seq
7	4.9	5.1	5.7	4.9	4.3	5.1		94.3	95.7	93.8	94.5	94.2	7	Bel936 Belgium Rep.seq
8	3.7	3.4	6.5	3.7	3.9	3.4	5.9		96.9	95.1	93.8	95.9	8	FraA4042 France Rep.seq
9	1.9	2.9	5.8	1.9	1.7	2.9	4.5	3.2		95.5	94.4	96.3	9	Ger1 Germany Rep.seq
10	4.8	4.6	6.6	4.8	4.5	4.6	6.5	5.1	4.7		93.7	95.9	10	NI7050 UK Rep.seq
11	5.9	5.3	0.0	5.9	6.3	5.3	5.7	6.5	5.8	6.6		93.9	11	PiCV-Zj1 China Rep.seq
12	4.1	3.4	6.4	4.1	4.1	3.4	6.0	4.2	3.8	4.2	6.4		12	USA93A USA Rep.seq
	1	2	3	4	5	6	7	8	9	10	11	12		

Fig. 2 Percent identity of 12 PiCV strains based on *Rep* protein amino acid sequences. Samples 1–6 are PiCV sequences obtained in this study. Samples 7–12 are PiCV sequences from GenBank

determine the clinical signs of illness [28, 29], and the initial PiCV infection may be overlooked.

There are currently no serological tests to diagnose PiCV, and the virus has not yet been grown *in vitro* [30]. Original diagnoses of PiCV infection were dependent on histopathological examination and electron microscopy, but recent diagnostic approaches have focused on detecting the specific PiCV DNA, and a number of PCR methods have been developed and applied [31–33]. The pairwise comparison of 12 complete PiCV genomes as well as *Cap* and *Rep* genes showed that regardless of where the strains were collected, the level of nt sequence identity was very similar, and differences in geographic origin play only a minor role in their genetic relationships. It is notable that the sequence similarity of *Rep* genes (93.8 %–100 %) was much higher than

that of *Cap* genes (79.1 %–100 %) among the PiCV genomes. It is well known that ORF V1 is located on the sense strand, while ORF C1 is located on the complementary strand [34]. The 5′ intergenic region is located between the start sites of the two ORFs and contains a highly conserved nucleotide sequence (TAGTATTAC), the initiation site of rolling-circle replication [35]. Thus, conserved nucleotide sequences and 5′ intergenic regions within the PiCV *Rep* genes would be suitable for inclusion in diagnostic PCR primers, while variable sequences within the *Cap* genes should be considered for phylogenetic studies and for selecting virus isolates for vaccine development [36].

Our study confirms that PiCV should be included as a member of the family *Circoviridae* based on the close relationship of PiCV strains to other circoviruses, the size

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12		
1	■	83.1	78.6	100.0	95.7	83.1	83.5	97.0	96.8	93.9	78.6	92.7	1	AHBZ Cap.seq
2	19.3	■	78.9	83.1	84.5	100.0	80.9	83.3	83.5	83.9	78.8	83.4	2	HBLF-E2 Cap.seq
3	25.2	24.9	■	78.6	79.8	78.9	80.9	79.1	79.8	79.9	100.0	79.7	3	JSNJ Cap.seq
4	0.0	19.3	25.2	■	95.7	83.1	83.5	96.9	96.8	93.9	78.6	92.7	4	NJPK-21 Cap.seq
5	4.4	17.4	23.6	4.4	■	84.5	84.9	96.8	96.5	97.0	79.8	94.3	5	SDDZ Cap.seq
6	19.3	0.0	24.9	19.3	17.4	■	80.9	83.3	83.5	83.9	78.8	83.4	6	SHWH-AB4 Cap.seq
7	18.7	22.0	22.1	18.7	16.9	22.1	■	84.7	84.9	84.6	80.8	85.4	7	Bel936 Belgium Cap.seq
8	3.1	19.0	24.6	3.1	3.2	19.0	17.2	■	98.5	94.6	79.0	93.7	8	FraA4042 France Cap.seq
9	3.2	18.7	23.6	3.2	3.6	18.7	16.9	1.5	■	95.0	79.8	93.7	9	Ger1 Germany Cap.seq
10	6.4	18.2	23.4	6.4	3.1	18.2	17.3	5.6	5.2	■	79.9	95.4	10	NI7050 UK Cap.seq
11	25.2	24.9	0.0	25.2	23.6	24.9	22.1	24.6	23.6	23.5	■	79.7	11	PiCV-Zj1 China Cap.seq
12	7.7	18.8	23.7	7.7	6.0	18.9	16.3	6.6	6.6	4.8	23.8	■	12	USA93A USA Cap.seq
	1	2	3	4	5	6	7	8	9	10	11	12		

(Left margin label: Divergence)

Fig. 3 Percent identity of 12 PiCV strains based on *Cap* protein amino acid sequences. Samples 1–6 are PiCV sequences obtained in this study. Samples 7–12 are PiCV sequences from GenBank

Percent Identity

|	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
1	■	88.6	88.1	100.0	97.2	90.9	90.2	97.0	97.8	95.2	88.1	95.0	56.5	59.5	55.3	64.5	54.2	41.3	41.4	42.1	41.4	41.9	32.5	1	AHBZ China.seq
2	12.4	■	87.2	88.6	89.3	97.5	86.9	88.8	89.1	88.8	87.2	88.9	55.7	58.4	53.4	62.9	52.8	40.6	40.5	41.5	40.6	41.5	31.4	2	HBLF-E2 China.seq
3	13.0	14.1	■	88.1	88.3	89.0	88.8	88.0	88.0	88.6	88.4	100.0	56.1	59.5	54.6	64.0	52.8	41.9	41.8	42.5	40.8	41.9	32.6	3	JSNJ China.seq
4	0.0	12.4	13.0	■	97.2	90.9	90.2	97.0	97.8	95.2	88.1	95.0	56.5	59.5	55.3	64.5	54.2	41.3	41.4	42.1	41.4	41.9	32.5	4	NJPK-21 China.seq
5	2.9	11.6	12.7	2.9	■	91.5	91.0	96.8	97.7	96.6	88.3	95.5	56.5	59.2	55.5	64.5	54.6	41.2	40.8	41.7	41.4	42.2	31.9	5	SDDZ China.seq
6	9.8	2.6	11.9	9.8	9.1	■	88.9	90.9	91.3	90.8	89.0	91.0	56.6	59.1	54.7	64.5	53.6	41.3	41.1	41.9	41.1	42.0	31.6	6	SHWH-AB4 China.seq
7	10.5	14.4	12.1	10.5	9.6	12.0	■	90.3	91.0	90.0	88.8	90.4	56.6	59.2	54.7	65.0	54.0	41.0	41.2	42.3	40.7	41.4	32.5	7	Bel936 Belgium.seq
8	3.1	12.1	13.0	3.1	3.3	9.7	10.4	■	97.8	95.4	88.0	95.2	56.2	59.2	55.1	64.5	54.2	41.7	41.3	41.9	40.9	41.6	32.4	8	FraA4042 France.seq
9	2.2	11.8	12.3	2.2	2.3	9.3	9.6	2.2	■	95.8	88.6	95.5	56.3	59.5	55.2	64.9	54.4	41.3	41.0	41.7	41.1	41.8	32.3	9	Ger 1 Germany.seq
10	4.9	12.1	12.6	4.9	3.5	9.9	10.6	4.7	4.4	■	88.4	96.1	56.2	59.0	55.6	64.3	54.1	41.7	41.2	41.8	40.8	41.6	31.7	10	NI7050 UK.seq
11	13.0	14.1	0.0	13.0	12.7	11.9	12.1	13.0	12.3	12.6	■	88.2	56.1	59.5	54.6	64.0	52.8	41.9	41.8	42.5	40.8	41.9	32.6	11	PiCV-zj1 China.seq
12	5.2	12.0	12.8	5.2	4.6	9.6	10.2	4.8	4.7	4.0	12.8	■	55.9	59.0	54.9	64.5	54.1	41.5	40.8	42.1	41.0	41.9	32.5	12	US93A USA.seq
13	65.1	67.1	66.2	65.1	65.1	65.0	65.0	65.9	65.6	65.9	66.2	66.6	■	68.8	55.0	60.5	53.7	40.9	40.4	41.7	38.6	39.2	33.1	13	CaCV Australia.seq
14	58.8	61.2	58.6	58.8	59.2	59.6	59.2	59.4	58.6	59.7	58.6	59.8	40.3	■	56.1	59.0	53.8	43.2	41.9	42.1	38.9	38.1	33.3	14	RaCV Australia.seq
15	68.5	73.3	70.3	68.5	68.0	70.0	69.9	69.0	68.7	67.9	70.3	69.4	69.0	66.2	■	53.8	57.4	40.4	41.2	41.5	40.3	40.7	30.2	15	BFDV Australia.seq
16	48.5	51.6	49.3	48.5	48.4	48.5	47.4	48.6	47.7	48.8	49.3	48.6	56.3	59.9	72.6	■	53.6	41.6	41.7	42.0	39.8	39.8	31.0	16	StCV Germany.seq
17	71.0	75.1	74.9	71.0	70.0	72.9	71.6	71.0	70.4	71.4	74.9	71.3	72.2	72.4	63.8	73.4	■	40.4	40.0	40.1	40.3	38.5	30.7	17	GuCV UK.seq
18	116.3	120.0	113.6	116.3	116.9	116.6	117.9	113.9	116.1	114.0	113.6	115.0	117.4	106.8	120.1	114.5	119.6	■	68.5	68.3	47.3	47.5	35.7	18	GoCV USA.seq
19	116.7	120.5	114.1	116.7	119.5	117.3	117.1	116.9	118.7	117.1	114.1	119.5	119.8	112.9	117.6	114.1	121.8	41.1	■	82.7	46.6	47.7	34.3	19	DuCV-LY0701 China.seq
20	112.7	115.1	110.5	112.7	114.6	113.2	111.6	113.4	114.6	113.5	110.5	112.4	113.1	112.1	115.7	112.9	121.7	41.3	19.7	■	47.0	48.4	33.9	20	DuCV-WF0701 China.seq
21	114.9	119.5	118.0	114.9	115.3	116.5	118.3	117.4	116.3	118.2	118.0	116.9	129.8	128.9	119.7	123.6	119.8	91.4	94.0	92.3	■	77.9	32.7	21	PCV1 Australia.seq
22	112.7	115.1	112.1	112.7	111.6	112.7	114.6	114.2	113.0	114.5	112.1	112.8	126.9	133.4	118.8	123.3	130.9	90.5	90.0	87.7	26.2	■	31.4	22	PCV2 Canada.seq
23	185.3	196.9	185.7	185.3	192.8	199.5	183.8	186.8	185.2	197.3	185.7	184.4	175.9	179.0	222.0	222.3	203.9	154.2	167.3	171.6	184.5	204.4	■	23	CAV-CUX-1 USA.seq
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		

(Left margin label: Divergence)

Fig. 4 Percent identity of 23 circovirus genomes. Samples 1–6 are PiCV strains obtained in this study. Samples 7–12 are PiCV strains and samples 13–23 are circovirus sequences from GenBank

of the virus, and associated pathologic features [37]. Further research on the family *Circoviridae* will likely identify more members of this interesting viral lineage. In this study, we included 10 members of the family *Circoviridae* whose genome sequences were available in GenBank, including nine circoviruses and one gyrovirus [38, 39]. Members of the genus *Circovirus* formed a group in the phylogenetic tree, and there were no discernible evolutionary patterns related to host differences or geographical distribution (Fig. 5). The two genera of the *Circoviridae* family, *Circovirus* and *Gyrovirus*, are reciprocally monophyletic according to these preliminary data (Fig. 4) and have a different evolution process. The 12 PiCV samples formed a clade and had a close relationship to each other regardless where they were collected.

PiCV infection affects healthy and diseased young pigeons and is thought to be responsible for immune suppression, resulting in complex and variable syndromes. This virus affects pigeons across the world, and outbreaks may have a devastating impact on poultry farming if the virus is left unchecked. Our study provides information

Fig. 5 Neighbor-joining tree of 23 circovirus genomes. "●"indicates PiCV genomes obtained in this study, "○" indicates PiCV sequences from GenBank. The 12 PiCV strains are within the shaded box
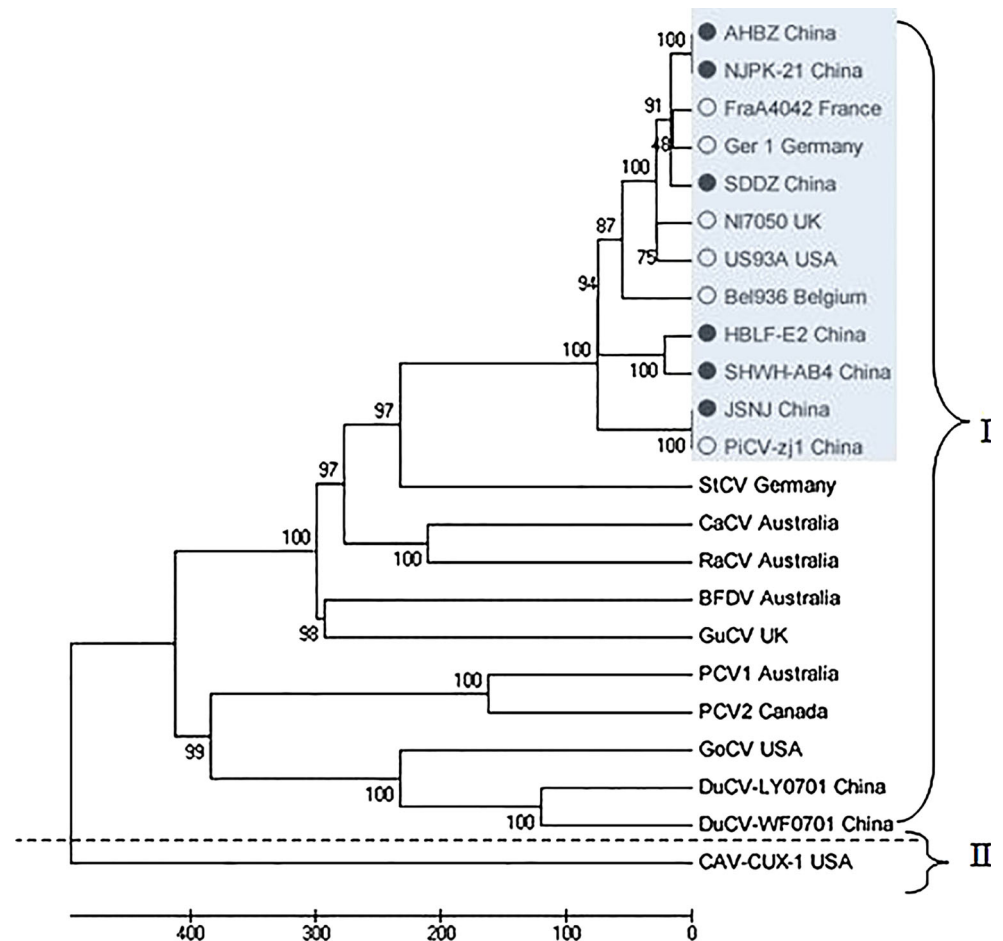
about the prevalence of PiCV in eastern China, reveals characteristics of PiCV genomes, and lays the foundation for further research about genetic variation and molecular evolutionary relationships in the family *Circoviridae*. Future research should aim to include more samples of PiCV from across its distribution area and should explore the pathogenic mechanism of PiCV and methods for culturing this virus.

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