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Emergence of canine parvovirus type 2c in domestic dogs and cats from Thailand

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Abstract

Canine parvovirus type 2 (CPV-2) is an important pathogen causing haemorrhagic enteritis in domestic dogs and wildlife worldwide. In early 2000, canine parvovirus type 2c (CPV-2c) was first reported and subsequently became a predominant subtype circulating in Europe and the Americas. CPV-2c has also been reported in Asia, including cases in China, India, Taiwan and Vietnam. However, CPV-2c has never been reported in Thailand. In this study, we conducted viral enteric disease surveillance in dogs and cats in Thailand during 2016–2018. During 20 months of surveillance, 507 rectal swab samples were collected from dogs (n = 444) and cats (n = 63) with and without clinical signs. The samples were examined for parvovirus by using VP2 gene-specific PCR for parvovirus. Our results showed that the positivity of canine parvovirus (CPV) was 29.95% and that of feline parvovirus (FPV) was 58.73%. In this study, we characterized 34 parvoviruses by VP2 gene sequencing. Moreover, two Thai-CPV-2 (Dog/CU-24 and Cat/CU-21) were characterized by whole genome sequencing. The phylogenetic results showed that Thai-CPV-2 had the highest nucleotide identities and clustered with Asian-CPV-2c but were in separate subclusters from the North American and European CPV-2c. Similarly, whole genome analyses showed that Thai-CPVs are closely related to Asian-CPV-2c, with unique amino acids at positions 297A, 324I, 370R and 426E. In summary, our results demonstrated the emergence of Asian-CPV-2c in dogs and cats in Thailand. Thus, the surveillance of CPV-2 in domestic dogs and cats should be further conducted on a larger scale to determine the dynamics of predominant variants and their distributions in the country and in the Southeast Asia region.

KEYWORDS
canine parvovirus, characterization, detection, emergence, Thailand

1 | INTRODUCTION

Canine parvovirus type 2 (CPV-2) is an important pathogen for domestic dogs and wildlife worldwide. CPV-2, a non-envelop, single-stranded DNA virus, belongs to the family Parvoviridae. CPV-2 causes acute haemorrhagic enteritis and myocarditis in dogs with high morbidity and frequent mortality (ranging 10%–90%). In 1977, it was first reported that CPV-2 arose from feline panleukopenia virus (FPV) with at least six coding nucleotide differences in the VP2 gene. CPV-2 can be further grouped into

three antigenic variants, including CPV-2a, CPV-2b and CPV-2c, based on unique amino acid residues at the positions 297 and 426 of VP2 (Buonavoglia et al., 2001). CPV-2a and CPV-2b were reported in 1979 and 1984, with unique amino acid residues as 426N and 426D, respectively. Both CPV-2a and CPV-2b variants are distributed worldwide and infect both dogs and cats but exhibit low pathogenicity in cats (Clegg et al., 2012). In 1990, CPV-2a and CPV-2b were replaced by two new variants of CPV-2a (CPV-2a-297A) and CPV-2b (CPV-2b-297A), with one unique amino acid substitution, S297A (Decaro et al., 2009). In 2000, CPV-2c was first reported in Italy with one substitution at the VP2 gene (D426E) (Buonavoglia et al., 2001). Recently, CPV-2c has been circulating predominantly in Europe and the Americas (Decaro & Buonavoglia, 2012). CPV-2c has also been reported in Asia, including cases in China, India, Taiwan and Vietnam (Chiang, Wu, Chiou, Chang, & Lin, 2016; Nakamura et al., 2004; Nandi, Chidri, Kumar, & Chauhan, 2010; Zhao et al., 2016). It has also been reported that CPV-2c can cause severe diseases in cats (Miranda, Parrish, & Thompson, 2014; Nakamura et al., 2001). In Thailand, CPV-2a and CPV-2b have been reported as major variants circulating in dogs (Phromnoi, Sirinarumitr, & Sirinarumitr, 2010), while CPV-2c has never been reported in the country. In this study, CPV-2c was detected in domestic dogs and cats during a viral enteric disease surveillance. This study is the first to report and characterize an emergence of Asian-CPV-2c in domestic dogs and cats in Thailand.

2 | MATERIALS AND METHODS

From September 2016 to April 2018, the centre of excellence for emerging and re-emerging infectious diseases in animals (CUEIDAs), Chulalongkorn University, conducted a viral enteric disease surveillance of domestic dogs and cats in Thailand. The surveillance was carried out in four provinces of Thailand under the animal use and care protocol # 1731074. Rectal swab samples were mainly collected from dogs and cats with acute haemorrhagic or watery diarrhoea, vomiting, fever and dehydration. During 20 months of surveillance, 507 rectal swab samples were collected from dogs (n = 444) and cats (n = 63) of young age (<1 year), adult (1–5 years) and older (>5 years) with vaccination history records. Of 444 canine samples, 366 samples from sick dogs and 78 from healthy dogs were collected. Of 63 feline samples, 60 samples from sick cats and three from healthy animals were collected. All samples were subjected to parvovirus identification by PCR specific to the VP2 gene, as previously described (Buonavoglia et al., 2001).

For parvovirus identification, viral DNA was extracted from rectal swab samples by using the QIAsymphony DSP viral/Pathogen mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The viral DNA was stored at −20°C until used. PCR assay for parvovirus identification was conducted as previously described (Buonavoglia et al., 2001). The oligonucleotide primers specific to the VP2 gene were Hfor: 5′-CAGGTGATGAATTGCTACA-3′ and Hrev: 5′-CATTTGGATAAACTGGTG GT-3′, located at positions 3556–3575 and 4166–4185 of CPV-2, respectively. In brief, PCR was performed in a final volume of 20 µl comprising 1 µl of DNA, 0.8 µM of each forward and reverse primer, 1× TopTaq Master Mix (Qiagen, Hilden, Germany), 1× CoralLoad, and distilled water. The PCR condition was set as initial denaturation step at 94°C for 3 min 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s and extension at 72°C for 1 min and final extension at 72°C for 7 min. The expected size of parvovirus positive amplified product was 611 bp. Identification of CPV2 antigenic variants was performed by using PCR-RFLP to differentiate CPV-2c and CPV-2a/CPV-2b variants. The PCR product size was 583 bp of the coding capsid protein VP2. Then, the PCR product was digested with enzyme Mbo II (New England Biolabs, USA) that selectively recognizes the restriction site "GAAGA" (nucleotide 4062–4066 of the VP2 encoding gene). The CPV-2c was digested into two fragments of 500 bp and 83 bp (Buonavoglia et al., 2001). The negative samples from CPV-2c PCR-RFLP assay were detected for CPV-2a and CPV-2b variants with specific primers (CPV-2abF/CPV-2abR and CPV-2bF/CPV-2bR) generating the product size of 681 bp and 427 bp, respectively (Pereira, Leal, & Durigon, 2007; Pereira, Monezi, Mehnert, D'Angelo, & Durigon, 2000) (Table S1). Concurrently, the CPV-2a/CPV-2b samples were confirmed by sequencing of the flanking region at amino acid position 426 to identify CPV-2a or CPV-2b variants.

For parvovirus characterization, we selected two parvoviruses (Dog/CU-24 and Cat/CU-21) for whole genome sequencing and the other 32 parvoviruses (CPV-2 = 21, FPV = 11) for VP2 gene sequencing. The criteria for selecting these 34 viruses for genetic characterization were based on epidemiological and demographic data, such as age of dog, date of isolation, breed and vaccination history. The selection criteria for the two viruses for whole genome sequencing were based on the representatives of CPV-2c from dogs (CU-24) and cats (CU-21). Parvovirus genome sequencing was conducted by using oligonucleotide primer sets previously described or new primer sets designed using the Primer 3 plus program (Table S1) (Buonavoglia et al., 2001; Koressaar & Remm, 2007; Untergasser et al., 2012). In brief, PCR was performed in a final volume of 30 µl comprising 2 µl of DNA, 0.4 µM of each forward and reverse primer, 1× TopTaq Master Mix, 1× CoralLoad, and distilled water. The PCR condition was set as initial denaturation at 94°C for 3 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s, extension at 72°C for 2 min and final extension at 72°C for 7 min. PCR products were then purified and sequenced (1st Base Laboratories Sdn Bhd, Malaysia). Nucleotide sequences were assembled by using SeqMan software v.5.03 (DNASTAR Inc., Madison, WI).

For genetic analysis, pairwise comparison was conducted by using MegAlign software v.5.03 (DNASTAR Inc.). In brief, the nucleotide sequences and deduced amino acids of Thai-CPV-2

and FPV were aligned with those of vaccine and reference strains of CPV2-a, CPV-2b, CPV-2c, CPV-2a-297A, CPV-2b-297A from the USA (CPV-13/1981, CPV-411b/1998, OH20219/2015), Japan (Y1), China (SC-02/2011), India (KolkataD5/2014), Indonesia (HCM14/2013), Italy (288-01/2001, 1-99/1999), Vietnam (HCM7/2013) and Thailand (KU14/2008). Genetic analysis for CPV-2 antigenic typing (VP2 at positions 297 and 426) and important amino acid determinants (VP2 at positions

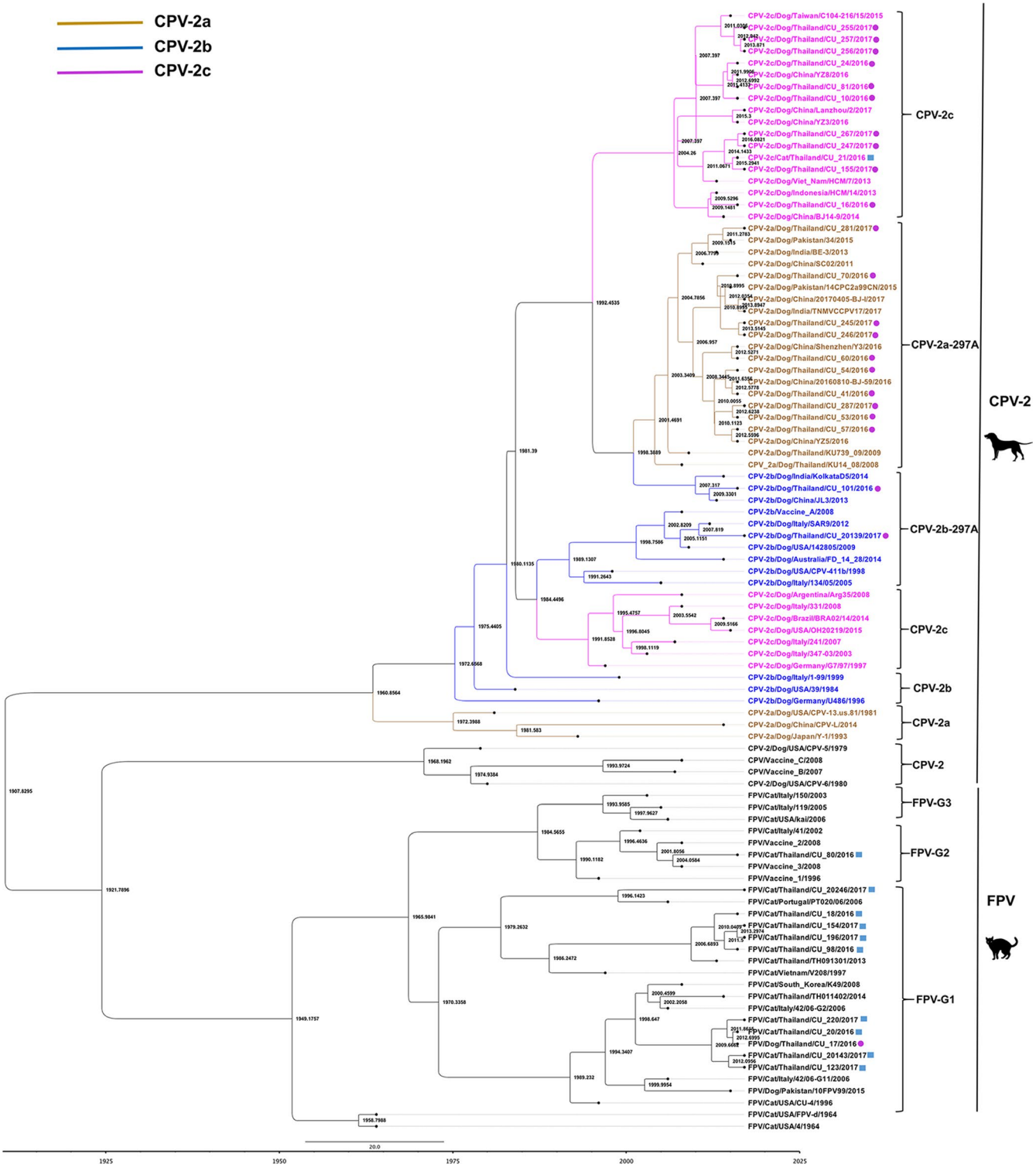

FIGURE 1 Phylogenetic tree of VP2 gene of canine parvovirus type 2 and feline parvovirus. Circles and squares represent Thai-CPV-2 and FPV, respectively. The phylogenetic tree was constructed by using the Beast program with Bayesian Markov-Chain Monte Carlo (BMCMC), with 10,000,000 generations and an average standard deviation of split frequencies <0.10. Values on branches represent times of most recent common ancestor (TMRCA) among CPV-2 antigenic types [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Association between age and clinical presentations of CPV-2 and FPV detection in this study

	Dogs		Cats	
	CPV-2 positive (%)		FPV positive (%)	
Age	Asymptomatic	Clinical sign	Asymptomatic	Clinical sign
Young (<1 year)	0/12 (0%)	91/198 (45.96%)	2/3 (66.67%)	28/47 (59.57%)
Adult (1–5 years)	3/63 (4.76%)	23/104 (22.12%)	0/0 (0%)	6/11 (54.55%)
Older (>5 years)	0/3 (0%)	16/64 (25.00%)	0/0 (0%)	1/2 (50.00%)
	3/78 (3.84%)	130/366 (35.52%)	2/3 (66.67%)	35/60 (58.33%)

TABLE 2 Association between vaccine history and clinical presentations of CPV-2 and FPV detection in this study

	Dogs		Cats	
	CPV-2 positive (%)		FPV positive (%)	
Vaccine history	Asymptomatic	Clinical sign	Asymptomatic	Clinical sign
Non-vaccination	0/67 (0%)	103/231 (44.59%)	2/3 (66.67%)	34/53 (64.15%)
Completed	3/11 (27.27%)	27/135 (20.00%)	0/0 (0%)	1/7 (14.29%)
	3/78 (3.85%)	130/366 (35.52%)	2/3 (66.67%)	35/60 (58.33%)

300, 305, 321, 323, 324, 370, 371, 375) was conducted by the alignment of VP2 by using MEGA v6.06 and MegAlign software v.5.03 (DNASTAR Inc.). For the phylogenetic analysis, the partial VP2 gene sequences of Thai-CPV-2 and FPV were analysed with those of reference viruses. Vaccine and reference viruses, including CPV-2-vaccine strains (n = 3), CPV-2 (n = 2), CPV-2a (n = 3), CPV-2b (n = 3), CPV-2c (n = 14), CPV-2a-297A (n = 11), CPV-2b-297A (n = 7), FPV vaccine (n = 3), FPV-G1 (n = 9), FPV-G2 (n = 1) and FPV-G3 (n = 3), were included in the phylogenetic analysis. The maximum clade credibility (MCC) tree of partial VP2 gene was constructed by BEAST 1.8 with the Bayesian Markov-Chain Monte Carlo (BMCMC) algorithm. A strict clock model with coalescent constant population and HKY with gamma 4 substitution were used as model parameters (Drummond, Suchard, Xie, & Rambaut, 2012). The Bayesian MCMC chain lengths were 10,000,000 generations, with sampling every 10,000 generations. The tree iteration was discharged with 10% of the chains as burn-in pattern by using a tree annotator, and the resulting MCC tree was drawn with FigTree software (v1.4.2) (Molecular evolution, phylogenetics and epidemiology, Edinburgh, Scotland, UK) (Figure 1). To determine the selective pressure on the partial VP2 (nucleotide positions 817–1314, amino acid positions 274–428), the ratio of non-synonymous (dN) to synonymous (dS) substitutions was estimated using Mixed Effects Model of Evolution (MEME) within the HyPhy software package (Murrell et al., 2012). The significance levels were set at p = 0.1. The values dN/dS > 1, dN/dS = 1 and dN/dS < 1 were used to define positive selection, neutral mutations, and negative selection, respectively. A phylogenetic tree was also constructed by using maximum-likelihood with bootstrap analysis of 1,000 replications using the MEGA v.6.06 program (Tamura, Dudley, Nei, & Kumar, 2007) (Figure S1).

3 | RESULTS

From September 2016 to April 2018, a viral enteric disease surveillance of domestic dogs and cats was conducted in four provinces of Thailand. Of 444 canine samples and 63 feline samples subjected to parvovirus identification, the positivity of CPV-2 in dogs was 29.95% (133/444) and that of FPV in cats was 58.73% (37/63), which were high in non-vaccinated animals (44.59%). Moreover, animals of young age (<1 year) were more frequently infected with CPV-2 (45.96%) (Tables 1 and 2). In this study, all samples were also examined for other important enteric viruses, including canine rotavirus (CRV) and canine coronavirus (CoV). We found coinfection of CPV-2 and CRV (n = 1) as well as CPV-2 and CoV (n = 22) in dogs. Additionally, coinfection of FPV and CoV was observed in two cats (data not shown).

In this study, we identified antigenic types of CPV-2 as CPV-2c (n = 62; 46.61%), CPV-2a (n = 68; 51.13%) and CPV-2b (n = 3; 2.26%) (Table S2). It is noted that both CPV-2c and CPV-2a were predominant variants and CPV-2c has never been reported in Thailand. In this study, we selected 34 parvoviruses for genetic characterization. For CPV-2, the viruses were subjected to VP2 gene (n = 21) and whole genome sequencing (n = 2; Dog/CU-24 and Cat/CU-21). For FPV, the viruses were subjected to VP2 gene sequencing (n = 11). The nucleotide sequences of the parvoviruses were submitted to the GenBank database under accession no. MH711880–MH711913 (Table 3). Pairwise comparisons of nucleotide and deduced amino acid sequences of Thai viruses were performed against those of vaccine and reference strains. Our results showed that the whole genomes of two Thai-CPV-2 (Dog/CU-24 and Cat/CU-21) had 99.90% nucleotide identity to each other and the highest nucleotide identities to Vietnam CPV-2c (99.60% at WG, 99.90% at VP2)

TABLE 3 Detailed descriptions of CPV-2 and FPV characterized in this study

Virus	Breed	Age of animal	Vaccine history	Clinical sign	Collection date	Location	Type of CPV/FPV	GenBank #
CPV								
Dog/Thailand/CU-41/2016	Mixed	2 years	C	Asymptomatic	Oct-16	Bangkok	CPV-2a-297A	MH711880
Dog/Thailand/CU-53/2016	Pomeranian	2 months	I	Diarrhoea	Oct-16	Bangkok	CPV-2a-297A	MH711881
Dog/Thailand/CU-54/2016	Yorkshire terrier	1 years	C	Diarrhoea	Oct-16	Bangkok	CPV-2a-297A	MH711882
Dog/Thailand/CU-57/2016	Pomeranian	2 months	I	Diarrhoea	Oct-16	Bangkok	CPV-2a-297A	MH711883
Dog/Thailand/CU-60/2016	Pomeranian	2 months	I	Diarrhoea	Oct-16	Bangkok	CPV-2a-297A	MH711884
Dog/Thailand/CU-70/2016	Siberian husky	4 months	I	Diarrhoea	Oct-16	Bangkok	CPV-2a-297A	MH711885
Dog/Thailand/CU-245/2017	Mixed	2 months	I	Diarrhoea	Apr-17	Bangkok	CPV-2a-297A	MH711886
Dog/Thailand/CU-246/2017	Beagle	2 months	I	Diarrhoea	Apr-17	Bangkok	CPV-2a-297A	MH711887
Dog/Thailand/CU-281/2017	Mixed	3 months	I	Diarrhoea	Sep-17	Bangkok	CPV-2a-297A	MH711888
Dog/Thailand/CU-287/2017	Mixed	1 year	I	Diarrhoea	Sep-17	Bangkok	CPV-2a-297A	MH711889
Dog/Thailand/CU-101/2016	Pekingese	2 months	I	Diarrhoea	Dec-16	Bangkok	CPV-2b-297A	MH711890
Dog/Thailand/ CU-20139/2017	Beagle	2 months	I	Diarrhoea	Nov-17	Bangkok	CPV-2b-297A	MH711891
Dog/Thailand/CU-10/2016	Beagle	2 years	C	Asymptomatic	Sep-16	Bangkok	CPV-2c	MH711892
Dog/Thailand/CU-16/2016	Shih Tzu	2 months	I	Diarrhoea	Sep-16	Bangkok	CPV-2c	MH711893
Dog/Thailand/CU-24/2016	Mixed	2 years	C	Asymptomatic	Oct-16	Bangkok	CPV-2c	MH711894[a]
Dog/Thailand/CU-81/2016	Chihuahua	2 months	I	Diarrhoea	Nov-16	Bangkok	CPV-2c	MH711895
Dog/Thailand/CU-155/2017	Pomeranian	6 months	I	Diarrhoea	Jan-17	Bangkok	CPV-2c	MH711896
Dog/Thailand/CU-247/2017	Jack Russell	2 months	I	Diarrhoea	Apr-17	Bangkok	CPV-2c	MH711897
Dog/Thailand/CU-255/2017	German Shepherd	2 months	I	Diarrhoea	Jun-17	N.Ratchasima	CPV-2c	MH711898
Dog/Thailand/CU-256/2017	German Shepherd	2 months	I	Diarrhoea	Jun-17	N.Ratchasima	CPV-2c	MH711899
Dog/Thailand/CU-257/2017	German Shepherd	2 months	I	Diarrhoea	Jun-17	N.Ratchasima	CPV-2c	MH711900
Dog/Thailand/CU-267/2017	Mixed	4 months	I	Diarrhoea	Jul-17	Tak	CPV-2c	MH711901
Cat/Thailand/CU-21/2016	Mixed	5 months	I	Diarrhoea	Oct-16	Bangkok	CPV-2c	MH711902[a]
FPV								
Cat/Thailand/CU-80/2016	Mixed	6 months	I	Diarrhoea	Nov-16	Bangkok	FPV-G2	MH711903
Cat/Thailand/CU-18/2016	Mixed	5 months	I	Diarrhoea	Sep-16	Bangkok	FPV-G1	MH711904
Cat/Thailand/CU-20/2016	Mixed	5 months	I	Diarrhoea	Sep-16	Bangkok	FPV-G1	MH711905
Cat/Thailand/CU-98/2016	Mixed	2 months	I	Diarrhoea	Dec-16	Bangkok	FPV-G1	MH711906
Cat/Thailand/CU-123/2017	Mixed	9 months	I	Asymptomatic	Jan-17	Chiang mai	FPV-G1	MH711907
Cat/Thailand/CU-154/2017	Mixed	3 months	I	Diarrhoea	Jan-17	Bangkok	FPV-G1	MH711908

(Continues)

TABLE 4 (Continued)

Virus	Breed	Age of animal	Vaccine history	Clinical sign	Collection date	Location	Type of CPV/FPV	GenBank #
Cat/Thailand/CU-196/2017	Mixed	1 year	I	Diarrhoea	Feb-17	Bangkok	FPV-G1	MH711909
Cat/Thailand/CU-220/2017	Mixed	3 months	I	Diarrhoea	Feb-17	Bangkok	FPV-G1	MH711910
Cat/Thailand/CU-20143/2017	Mixed	2 months	I	Diarrhoea	Nov-17	Bangkok	FPV-G1	MH711911
Cat/Thailand/CU-20246/2018	Mixed	5 months	I	Diarrhoea	Jan-18	Bangkok	FPV-G1	MH711912
Dog/Thailand/CU-17/2016	Labrador retriever	13 years	C	Diarrhoea	Sep-16	Bangkok	FPV-G1	MH711913

Note. [a]Whole genome sequence.

(Table S3). Within Thai-CPV-2, the VP2 gene is diverse with nucleotide identities of 99.80%–100% (CPV-2c), 99.00%–99.20% (CPV-2b-297A) and 98.80%–99.00% (CPV-2a-297A) (Table S4). In this study, the overall dN/dS ratio for the partial VP2 of CPV-2 and FPV was lower than 1 (0.296, 0.032), implying that the gene was under negative selection or purifying selection as the main evolutionary force.

Phylogenetic analysis of the VP2 gene from Thai-CPV-2 showed that the viruses were clustered with CPV-2c, CPV-2a-297A and CPV-2b-297A. The phylogenetic analysis indicated that Thai-CPV-2c was closely related to VietNam-HCM7, Chinese-YZ-8, BJ14-9, Taiwan-C104 and Indonesia-HCM but was in separate subclusters from the North American and European CPV-2c (Figure 1 and Figure S1). Based on the MCC tree, the Asian-CPV-2c was estimated to separate from CPV-2C of America and Europe since 1981. While, Thai-CPV-2c was started to evolved from other Asian-CPV-2c viruses (China, Taiwan, Vietnam and Indonesia) since 2004. The estimated nucleotide substitution rate of the partial VP2 was 1.1905×10^{-4} substitutions per site per year. 95% highest posterior densities (HPD) was 6.9511×10^{-5}–1.6877×10^{-4}). It is noted that the new variant CPV-2b-297A (n = 2) was clustered in a separate group in which one isolate (Dog/CU-20139) was closely related to the vaccine strain (CPV-2b/Vaccine), suggesting a virus of vaccine origin. The phylogenetic analysis of the VP2 gene of FPV was also performed, showing that Thai-FPV was predominantly clustered with FPV-G1 (n = 10), including one canine isolate (Dog/CU-17). In contrast, one Thai-FPV (Dog/CU-80) was grouped in a distinct cluster (G2) with FPV vaccine strains (Figure S1). It is interesting to note that one dog isolate was clustered with FPV-G1, suggesting FPV infection in a dog.

Genetic analyses of the genomes of Thai-CPV-2 and FPV were also conducted (Table 4). CPV-2a, CPV-2b and CPV-2c variants were determined by genetic differences at VP2 position 426 as Asn (N), Asp (D) and Glu (E), respectively (Martella, Decaro, & Buonavoglia, 2006). In this study, the new variants CPV-2a-297A and CPV-2b-297A, had unique amino acids at positions 297A, 426N and 426D, which were also observed in reference viruses. Similarly, Thai-CPV-2c contained unique amino acids at positions 297A and 426E, which were observed in reference CPV-2c. It is important to note that unique amino acid substitutions at positions Y324I and Q370R were only observed in the Asian strain CPV-2c (VietNam-HCM7, Chinese-YZ-8, BJ14-9, Taiwan-C104 and Indonesia-HCM), including Thai-CPV-2c, but were not observed in American and European CPV2-c (Table 4 and Figure 2).

4 | DISCUSSION

To our knowledge, this study is the first to report CPV-2c in dogs and cats in Thailand. The infected animals showed clinical signs of acute haemorrhagic or watery diarrhoea. In this study, the positivity of CPV-2 in dogs was 29.95% and that of FPV in cats was 58.73%, which were high in non-vaccinated animals.

TABLE 4 Genetic analysis of deduced amino acids of Thai-CPV-2 and FPV in comparison to those of vaccine and reference strains

Strain	Accession number	Year	Country	Amino acid position of VP2 gene										Type
				Typing	Important amino acids									
				297	426	300[a]	305[a]	321	323[b]	324	370[c]	371	375	
Reference CPV														
CPV-2/Dog/USA/CPV-5/1979	EU659116	1979	USA	S	N	A	D	N	N	Y	Q	A	N	CPV-2
CPV-2/Dog/USA/CPV-6/1980	EU659117	1980	USA	S	N	A	D	N	N	Y	Q	A	N	CPV-2
CPV-2/Vaccine B (Nobivac;Intervet)	FJ197846	2007	South Korea	S	N	A	D	N	N	Y	Q	A	N	CPV-2/Vaccine
CPV-2/Vaccine C (Vaccine06;Merial)	FJ222822	N/A	N/A	A	D	G	Y	K	N	Y	Q	A	D	CPV-2/Vaccine
CPV-2a/Dog/USA/CPV-13/1981	EU659118	1981	USA	S	N	G	Y	N	N	Y	Q	A	D	CPV-2a
CPV-2a/Dog/Japan/Y1/xxxx	D26079	N/A	Japan	S	N	G	Y	N	N	Y	Q	A	D	CPV-2a
CPV-2a/Dog/Thailand/KU14/2008	GQ379043	2008	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2a/Dog/China/SC02/2011	JX660690	2011	China	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2b/Dog/Italy/1-99/1999	MF177226	1999	Italy	S	D	G	Y	N	N	Y	Q	A	D	CPV-2b
CPV-2b/Dog/USA/CPV-411b/1998	EU659121	1998	USA	A	D	G	Y	N	N	Y	Q	A	D	CPV-2b-297A
CPV-2b/Dog/India/KolkataD5/2014	KP071953	2014	India	A	D	G	Y	N	N	I	Q	A	D	CPV-2b-297A
CPV-2b/Vaccine A (Duramune;Fort Dodge)	FJ222822	N/A	N/A	A	D	G	Y	K	N	Y	Q	A	D	CPV-2b/Vaccine
CPV-2c/Dog/Italy/288-01/2001	MF177239	2001	Italy	A	E	G	Y	N	N	Y	Q	A	D	CPV-2c
CPV-2c/Dog/USA/OH20219/2015	MF457594	2015	USA	A	E	G	Y	N	N	Y	Q	A	D	CPV-2c
CPV-2c/Dog/Vietnam/HCM/7/2013	LC214969	2013	Vietnam	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Indonesia/HCM/14/2013	LC216909	2013	Indonesia	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Taiwan/C104-216/2015	KX421787	2015	Taiwan	A	E	G	Y	N	N	I	R	A	D	CPV-2c
This study: CPV														
CPV-2a/Dog/Thailand/CU 41/2016	This study	2016	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2a/Dog/Thailand/CU 53/2016	This study	2016	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2a/Dog/Thailand/CU 54/2016	This study	2016	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2a/Dog/Thailand/CU 57/2016	This study	2016	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2a/Dog/Thailand/CU 60/2016	This study	2016	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2a/Dog/Thailand/CU 70/2016	This study	2016	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2a/Dog/Thailand/CU 245/2017	This study	2017	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2a/Dog/Thailand/CU 246/2017	This study	2017	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2a/Dog/Thailand/CU 281/2017	This study	2017	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A

(Continues)

TABLE 4 (Continued)

Strain	Accession number	Year	Country	Amino acid position of VP2 gene										
				Typing	Important amino acids									
				297	426	300[a]	305[a]	321	323[b]	324	370[c]	371	375	Type
CPV-2a/Dog/Thailand/CU 287/2017	This study	2017	Thailand	A	N	G	Y	N	N	I	Q	A	D	CPV-2a-297A
CPV-2b/Dog/Thailand/CU 101/2016	This study	2016	Thailand	A	D	G	Y	N	N	I	Q	A	D	CPV-2b-297A
CPV-2b/Dog/Thailand/CU 20139/2017	This study	2017	Thailand	A	D	G	Y	K	N	Y	Q	A	D	CPV-2b-297A
CPV-2c/Dog/Thailand/CU 10/2016	This study	2016	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Thailand/CU 16/2016	This study	2016	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Thailand/CU 24/2016	This study	2016	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Thailand/CU 81/2016	This study	2016	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Thailand/CU 155/2017	This study	2017	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Thailand/CU 247/2017	This study	2017	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Thailand/CU 255/2017	This study	2017	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Thailand/CU 256/2017	This study	2017	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Thailand/CU 257/2017	This study	2017	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Dog/Thailand/CU 267/2017	This study	2017	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c
CPV-2c/Cat/Thailand/CU 21/2016	This study	2016	Thailand	A	E	G	Y	N	N	I	R	A	D	CPV-2c[a]
Reference FPV														
FPV/Cat/USA-4/1964	EU659112	1964	USA	S	N	A	D	N	D	Y	Q	A	D	
FPV/Cat/USA/kai/2006	EU659115	2006	USA	S	N	A	D	N	D	Y	Q	A	D	
FPV/Cat/Italy/42/06-G2/2006	EU498698	2006	Italy	S	N	A	D	N	D	Y	Q	A	D	
FPV/Cat/Thailand/TH011402/2014	KT357494	2014	Thailand	S	N	A	D	N	D	Y	Q	A	D	
FPV/Dog/Pakistan/10FPV99/2015	MF182903	2015	Pakistan	S	N	A	D	N	D	Y	Q	A	D	
FPV/Vaccine 1 (PLI-IV)	D88287	N/A	N/A	S	N	A	D	N	D	Y	Q	A	D	
FPV/Vaccine 2 (Purevax;Merial)	EU498680	N/A	N/A	S	N	A	D	N	D	Y	Q	A	D	
FPV/Vaccine 3 (Felocell;Pfizer)	EU498681	N/A	N/A	S	N	A	D	N	D	Y	Q	A	D	
This study: FPV														
FPV/Cat/Thailand/CU 80/2016	This study	2016	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G2
FPV/Cat/Thailand/CU 18/2016	This study	2016	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1
FPV/Cat/Thailand/CU 20/2016	This study	2016	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1
FPV/Cat/Thailand/CU 98/2016	This study	2016	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1
FPV/Cat/Thailand/CU 123/2017	This study	2017	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1

(Continues)

TABLE 4 (Continued)

Strain	Accession number	Year	Country	Amino acid position of VP2 gene									Type
				Typing	Important amino acids								
				297	426	300[a]	305[a]	321	323[b]	324	370[c]	371	375	
FPV/Cat/Thailand/CU 154/2017	This study	2017	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1
FPV/Cat/Thailand/CU 196/2017	This study	2017	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1
FPV/Cat/Thailand/CU 220/2017	This study	2017	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1
FPV/Cat/Thailand/CU 20143/2017	This study	2017	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1
FPV/Cat/Thailand/CU 20246/2017	This study	2017	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1
FPV/Dog/Thailand/CU 17/2016	This study	2016	Thailand	S	N	A	D	N	D	Y	Q	A	D	FPV-G1[b]

Notes. N/A:notavailable.

[a]A300G, D305Y amino acid residue related to the adaptation of the CPV variants to the feline host (Ikeda et al., 2000; Truyen, Evermann, Vieler, & Parrish, 1996). [b]D323N amino acid residue related to specific canine host (binding with canine receptor (TfR) (Chang, Sgro, & Parrish, 1992; Govindasamy, Hueffer, Parrish, & Agbandje-McKenna, 2003). [c]Q370R amino acid residue related to host range, novel Asian variant (Govindasamy et al., 2003).

This study also showed that CPV-2 was predominantly detected in dogs of young age (<1 year). These results were similar to the previous report of CPV-2 in puppies in Thailand (Sakulwira, Vanapongtipagorn, Theamboonlers, Oraveerakul, & Poovorawan, 2003). It is important to note that CPV-2c could also be isolated from cats. Similar observations were also reported in other studies (Miranda et al., 2014; Nakamura et al., 2001). One FPV infection in a dog was observed, as also seen in a previous study of FPV infection in sick dogs in Pakistan in 2018 (Ahmed et al., 2018).

Nucleotide and amino acid comparison showed that the whole genomes of two Thai-CPV-2 strains had 99.90% nucleotide identity to each other and had highest nucleotide identities to Asian-CPV-2c from Vietnam. Similar studies reported Asian-CPV-2c in China and Taiwan (Chiang et al., 2016; Guo et al., 2013). Phylogenetic analysis showed that Thai-CPV-2c is closely related to Asian-CPV-2c, including VietNam-HCM7, Chinese-YZ-8, BJ14-9, Taiwan-C104 and Indonesia-HCM. These viruses were in separate subclusters from North American and European CPV-2c. Our analysis suggested that the estimated time of the most recent common ancestor of Thai-CPV-2c subclusters was 2004 (Figure 1). The substitution rate of parvovirus in this study was in agreement with other studies (1.2×10^{-4}–2.2×10^{-4} substitutions per site per year) (Hoelzer, Shackelton, Parrish, & Holmes, 2008; Pereira et al., 2007; Shackelton, Parrish, Truyen, & Holmes, 2005). Moreover, our data indicated that parvovirus (which is DNA virus) has high genomic substitution rate similar to other RNA viruses at approximately 10^{-4} substitutions per site per year (Duffy, Shackelton, & Holmes, 2008). Whole genome analysis indicated that Thai-CPVs are closely related to Asian-CPV-2c with unique amino acids at position 297A, 370R and 426E of VP2, suggesting predominant Asian-CPV-2c in the country. It is also noted that unique amino acid substitutions at positions Y324I and Q370R were only observed in Asian strains of CPV-2c. These unique amino acids (370R) might relate to receptor-binding properties, suggesting species preference. Recent observations have also been reported in China and Taiwan (Chiang et al., 2016; Guo et al., 2013).

The identification of several types of CPV2 (CPV-2c, new variant CPV-2a-297A, and new variant CPV-2b-297A) demonstrates diversity of CPV2 in Thailand. CPV-2c is an emerging variant in the country and the Southeast Asia region. These findings will stimulate concern regarding whether currently used canine parvovirus vaccines will provide full protection against the new variant, Asian-CPV-2c. In summary, our results demonstrated the emergence of the new variant Asian-CPV-2c in dogs and cats in Thailand. Since cats can be infected with CPV-2c, dogs can also be infected with FPV. Thus, veterinary practitioners should focus more attention on both CPV and FPV infections, especially interspecies transmission. In Thailand, the surveillance of CPV and FPV should be further conducted on a larger scale to determine the dynamics of predominant variants and their distributions. This information will aid early diagnosis and the development of future strategies for domestic animal vaccination.

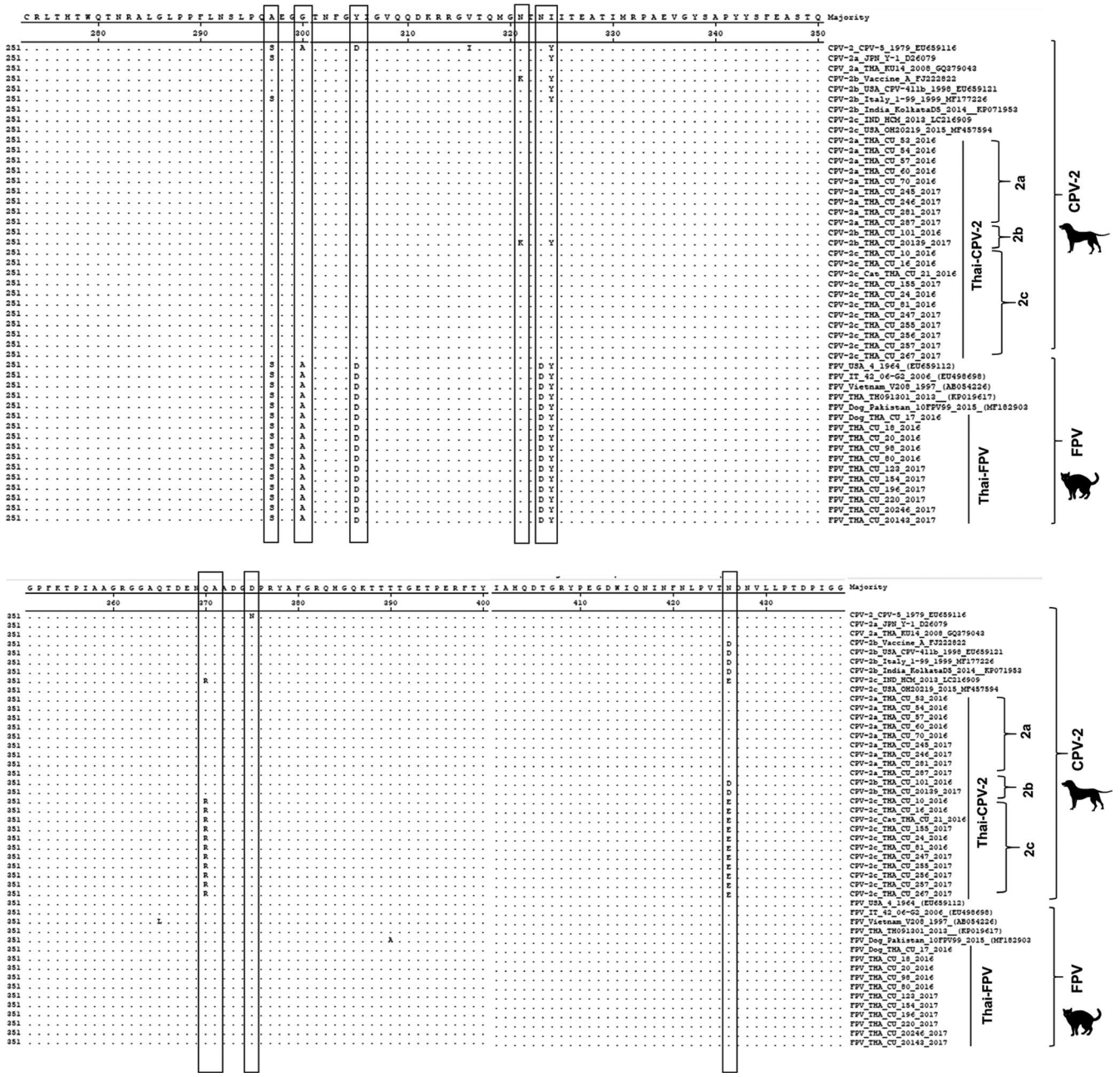

FIGURE 2 Amino acid alignment of VP2 protein of CPV-2. Dots represent matched amino acid residues. Open boxes indicate amino acid substitutions

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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