

Long-term surveillance of bat coronaviruses in Korea: Diversity and distribution pattern

Van Thi Lo^{1,2} | Sun-Woo Yoon^{1,2}  | Ji Yeong Noh³ | Youngji Kim¹ | Yong Gun Choi⁴ | Dae Gwin Jeong^{1,2}  | Hye Kwon Kim³ 

¹Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea

²Bio-Analytical Science Division, Korea University of Science and Technology (UST), Daejeon, Korea

³Department of Microbiology, College of Natural Sciences, Chungbuk National University, Cheongju, Korea

⁴The Korean Institute of Biospeleology, Daejeon, Korea

Correspondence

Hye Kwon Kim, Department of Microbiology, College of Natural Sciences, Chungbuk National University, Cheongju, Korea.
Email: khk1329@chungbuk.ac.kr

Dae Gwin Jeong, Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea.
Email: dgjeong@kribb.re.kr

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Abstract

Bats harbour diverse coronaviruses (CoVs), some of which are associated with zoonotic infections, as well as inter-species transmission. In this study, a total of 512 bat faecal samples from the bat habitats at different geographical locations in South Korea were investigated between 2016 and 2019. Seventy-eight samples were positive for coronaviruses (15.2%), comprising 68 alphacoronaviruses (13.3%) and 10 betacoronaviruses (2.0%). The positive rates tended to increase during the awakening (April) period. Notably, betacoronaviruses were only found in the site where *Rhinolophus ferrumequinum* was the major species of bats, and were related to SARS- and MERS-related CoVs identified in China and South Korea, respectively. No betacoronaviruses were closely related to SARS-CoV-2 in this study. Alphacoronaviruses were detected in the sites where *Hypsugo alaschanicus*, *Miniopterus fuliginosus*, *Miniopterus schreibersii*, *Rhinolophus ferrumequinum*, *Myotis bombinus*, *Myotis macrodactylus* and *Myotis petax* were found to be the major bat species. Furthermore, alphacoronaviruses had higher genetic diversity than betacoronaviruses and had a wider distribution in Korea. Considering that different bat species are co-roosting in crowded conditions in the same habitat, the diverse coronaviruses in Korean bats are likely to undergo cross-species transmission events due to the richness in host species. Therefore, continuous monitoring should be performed, especially at the awakening time of the hibernating bats in the habitats where diverse bat species co-roost, to better understand the evolution of coronaviruses in bats.

KEYWORDS

alphacoronavirus, bats, coronavirus, diversity, host sharing, Korea

1 | INTRODUCTION

Coronaviruses (CoVs) are the largest groups of positive-sense, single-stranded RNA viruses belonging to the *Coronaviridae* family. CoVs are divided into four genera, alpha-, beta-, gamma- and delta-coronaviruses (de Groot et al., 2011). Many species of CoVs exist

among mammals and birds. Alpha- and betacoronavirus have been reported only in mammals so far and can cause respiratory and gastrointestinal diseases with high mortality rates, for example transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhoea virus (PEDV) in young pigs, severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle East Respiratory syndrome coronavirus (MERS-CoV) in humans (Brian & Baric, 2005; Lee et al., 2003; Lin, Saif, Marthaler, & Wang, 2016; Zaki, Van Boheemen, Bestebroer, Osterhaus, & Fouchier, 2012).

Van Thi Lo, Sun-Woo Yoon and Ji Yeong Noh contributed equally.

Bats, a diverse group of mammals in the order Chiroptera, are not only mammals with the capability of powered flight but also the second-largest order among mammals and distributed worldwide (Wang & Anderson, 2019). Bats are known as the primary reservoir for viruses, such as lyssaviruses, paramyxoviruses and filoviruses. Bats are also recognized as a natural reservoir host of the coronaviruses (Allocati et al., 2016). SARS-CoV and MERS-CoV are suggested to have originated from bats, as palm civets and dromedary camel are intermediate hosts, respectively (Azhar et al., 2014; Cui, Li, & Shi, 2019). Swine enteric alphacoronavirus (SeACoV), also known as swine acute diarrhoea syndrome coronavirus (SADS-CoV), was genetically most closely related to HKU2-CoV and was found in bats. These viruses have originated from a common ancestor (Pan et al., 2017; Zhou et al., 2018).

The newly emerging coronavirus (also referred to as SARS-CoV-2) causing acute respiratory illnesses, such as pneumonia (COVID-19), was first identified in late December 2019 in Wuhan, China, and has emerged as a pandemic (<https://www.who.int/emergencies/diseases/novel-coronavirus-2019>). Bats were proposed as the most likely natural reservoir for SARS-CoV-2 even though the transmission from bats to human is still unknown (Zhou et al., 2020).

Since the emergence of SARS-CoV in 2003 and MERS-CoV in 2012, several studies have shown that bat CoVs are found in North and South America, Europe, Africa, Australia and Asia (Gouilh et al., 2018; August, Mathews, & Nunn, 2012; Carrington et al., 2008; De Sabato et al., 2019; Dominguez, O'Shea, Oko, & Holmes, 2007; Lazov et al., 2018; Liang et al., 2017; Lin et al., 2017; Rizzo et al., 2017; Smith et al., 2016; Tang et al., 2006; Tao et al., 2012; Wacharapluesadee et al., 2015; Watanabe et al., 2010; Woo et al., 2006). In South Korea, SARS-like and MERS-like coronaviruses were reported in bat faeces since 2015 (Kim et al., 2016). Another group also conducted surveillance of coronaviruses in bats in 2016 and showed that these were genetically similar to bat betacoronaviruses identified in China (Lee et al., 2018). However, long-term coronavirus surveillance has not been previously described in South Korea and the surrounding islands. The aim of the present study was to identify genetic characteristics of coronaviruses circulating in bats in South Korea. Furthermore, we also focus on describing the distribution and seasonal pattern of bat coronaviruses in Korea between 2016 and 2019.

Here, we collected 512 samples of bat faeces from 2016 to 2019 in Korean bat species. Phylogenetic analyses were performed under a Bayesian statistical framework implemented in BEAST.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

To avoid animal disturbance, the study was focused on the analysis of guano collected beneath bat roosting sites/colonies in Korea. This sampling design did not require ethical approval for the study. The authors confirm that the ethical policies of the

journal, as noted on the journal's author guidelines page, have been adhered to.

2.2 | Study sites and samples collection

Between 2016 and 2019, we obtained 512 faecal samples ($n_{2016} = 135$, $n_{2017} = 108$, $n_{2018} = 108$, $n_{2019} = 161$), which were collected from the bat habitats in 10 provinces and islands of South Korea (total of 111 sampling sites). The bat habitats were randomly chosen by the expert from the Korean Institute of Biospeleology among caves, abandoned mines, and under a bridge where bats inhabited. All the sampling locations were close to human settlements.

We sampled faecal pellets directly on the cave floor with sterile swabs. Fresh faecal samples were preferred. The faecal sample (0.3 g of faeces) was immediately placed into transport medium (1:10 wt/vol)(Universal Transport Medium, Noble Biosciences™). The samples were transported to the laboratory and ultimately stored at -80°C until analysis.

The accurate sampling locations were carefully recorded by noting the place name, latitude and longitude.

2.3 | Coronavirus detection and sequencing

All samples were cleared by centrifugation at $3,000 \times g$ for 15 min at 4°C . RNA was extracted by TRIzol LS (Invitrogen) from 250 μl of each sample supernatant and eluted in 40 μl DEPC water. RNA was extracted according to manufacturer's instructions. cDNA was synthesized with the M-MLV reverse transcriptase kit (Invitrogen) using random hexamer following the manufacturer's protocol. Coronaviruses were detected by a pan-coronavirus nested PCR specific for the RNA-dependent RNA polymerase (RdRp) sequences, as described previously (Chu et al., 2011). Briefly, the initial PCR reaction was made with 2 μl of cDNA, 2 \times AccuPower® PCR Master Mix (Bioneer, USA), 1 μM of each primer and nuclease-free water in a total volume of 20 μl . The first PCR used Chu-RdRp-N1-F (5'-GGKTGGGAYTAYCCAARTG-3') and Chu-RdRp-N1-R (5'-TGYTGTWRCARAAYTCRTG-3') primers. The PCR reaction mixture was made with 1 μl of the first PCR reaction, 2 \times AccuPower® PCR Master Mix (Bioneer), 1 μM of each primer and nuclease-free water in a total volume of 20 μl . The second nested PCR used Chu-RdRp-N2-F (5'-GGTTGGGACTATCCTAAGTGTGA-3') and Chu-RdRp-N2-R (5'-CCATCATCAGATAGAATCATCAT-3') primers and amplified a 440 bp product within the first PCR. The same thermal conditions were applied for both PCR: 95°C for 10 min; then 40 cycles of 95°C for 30 s, 48°C for 30 s and 72°C for 40 s; followed by a final extension step of 72°C for 5 min. The PCR products were revealed by electrophoresis on 1.5% agarose gels and were gel purified using QIAquick Gel Extraction kit (Qiagen). The PCR products were submitted to the Cosmogenetech company in Seoul, Korea for sequencing.

2.4 | Distribution and seasonal pattern analysis

The geocoding of each sampling site was plotted using the Google Maps API (Application Programming Interface) with the 'ggmap' R package based on obtained latitude and longitude for each sample. For seasonal pattern analysis, the percentage of positive coronavirus samples were recorded by the month in each year. A box plot for each month in the year within the surveillance time frame was plotted using the 'boxplot' function. Both analyses were conducted in R software (version 3.5.1).

2.5 | Phylogenetic analysis

Nucleotide sequences were aligned using ClustalW software implemented in BioEdit (version 7.1.9). Each sequence was trimmed to where both forward and reverse reads were in 100% agreement with the consensus sequence. Preliminary phylogenetic analyses using the Maximum Likelihood method based on the Tamura-Nei model were conducted. The main phylogenetic analyses were performed under a Bayesian statistical framework implemented in BEAST (version 1.10.4) (Drummond & Rambaut, 2007), using the best-fit model according to the corrected Bayesian Information Criterion (BIC) obtained in jModelTest 2 (Darriba, Taboada, Doallo, & Posada, 2012). The general time-reversible (GTR) model of substitution with a gamma distribution (GTR + G + I) and the coalescent model (constant size) was used in BEAST. Phylogenetic trees were displayed in FigTree (version 1.4.4).

2.6 | Biomolecular species identification

As the faecal sample was collected from caves which harbours multiple bat species, total DNA extraction was performed only for PCR-positive samples starting from the original suspensions using the QIAamp DNA

Mini kit (Qiagen), and following the manufacturer protocol. To confirm species identification, PCR was performed using specific primers for the *Cytochrome b gene* (*Cytb*) as previously described (Schlegel et al., 2012). PCR products were visualized on 1% agarose gels, purified using the QIAquick Gel Extraction kit (Qiagen) and sequenced as above.

Species identification was conducted by comparing the obtained sequences to available reference sequences (BLAST alignment, NCBI web site).

Accession number(s). The sequence data have been submitted to the GenBank databases under accession numbers MK991901 to MK991951.

3 | RESULTS

3.1 | Virus detection

In total, 512 samples were tested for the presence of coronavirus by nested RT-PCR detection of an RNA-dependent RNA polymerase (RdRp) gene fragment (Chu et al., 2011). Coronaviruses were detected in 15.2% (78/512) of samples obtained from the bat habitats in 8 of 9 provinces and islands of Korea where the most prevalent bat species were found to be: *Hypsugo alaschanicus* (1), *Miniopterus fuliginosus* (1), *Miniopterus schreibersii* (18), *Myotis bombinus* (1), *Myotis marcodactylus* (16), *Myotis petax* (5) and *Rhinolophus ferrumequinum* (36).

3.2 | Distribution and seasonal pattern of bat coronaviruses

A map showing sampling sites is presented in Figure 1, based on the coronavirus-positive sampling locations. From the total of 111 sampling sites, there are six sites belonging to four mainland provinces where betacoronaviruses were found, whereas alphacoronaviruses

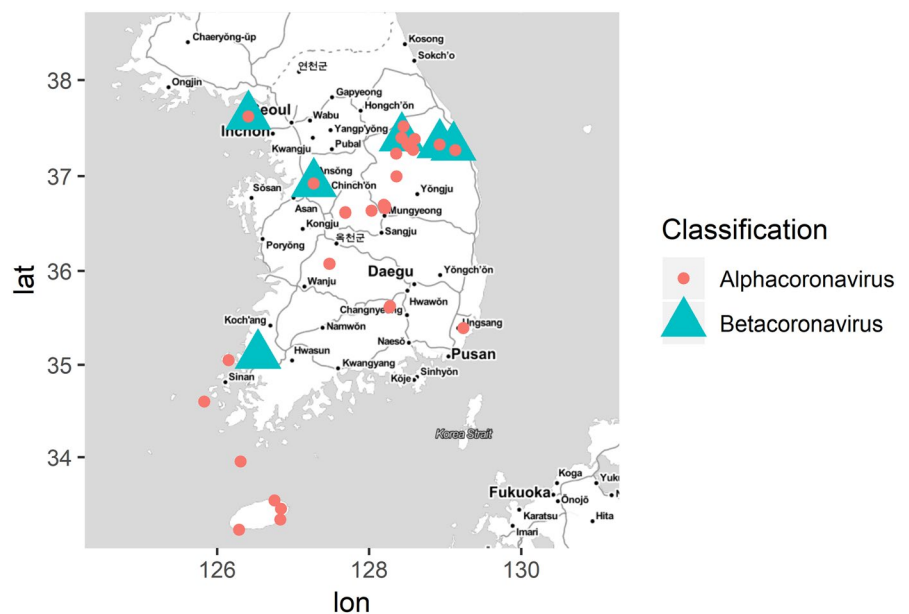


FIGURE 1 Map of geographical distribution of coronaviruses investigated in this study. Alphacoronavirus and betacoronavirus are depicted with red circle and green triangle, respectively. The map was built using ggmap and ggplot2 packages on R version 3.5.1

were detected at 25 sites in both mainland and island sites (Jeju Island and Island of Jeonam).

The seasonal pattern of bat coronavirus distribution during the study period between 2016 and 2019 is shown in Figure 2. Overall, the average and amplitude of the positivity rate fluctuated for each month over four years. Looking at the median, it seems clear that April exhibited relatively higher viral shedding rate, compared to winter (around January to March).

3.3 | Phylogenetic analysis

Phylogenetic analysis based on the nucleotide sequences of the partial RdRp gene (279 nt) in BEAST (v1.10.4) showed that the detected coronaviruses belonged to the genera Alphacoronavirus (13.3%: 68/512) and Betacoronavirus (2.0%: 10/512).

Alphacoronaviruses found at the site where seven bat species were predominant, namely *Hypsugo alaschanicus*, *Miniopterus fuliginosus*, *Miniopterus schreibersii*, *Myotis bombinus*, *Myotis macrodactylus*, *Myotis petax* and *Rhinolophus ferrumequinum*. These 68 alphacoronaviruses were differently clustered. The sequences sharing $\geq 90\%$ nucleotide identity are collapsed into the same clade with $\geq 95\%$ of the posterior probability support. The alphacoronaviruses found in this study were divided into eight clades: K1, K2, K3, K4, HKU6, HKU7, HKU8 and HKU10-related (Figure 3). All sequences of K2 and K3 clades show high identity to BtCoV/Myotis emarginatus/LUX (90.4 to 91.5% nucleotide sequence identity) found in Luxembourg and BtCoV/A910/2005 (95.2 to 96.6% nucleotide sequence identity) found in Hong Kong, respectively. Only B19-69 (K4 clade) clustered with PEDV, sharing 72.3% nucleotide sequence identity. Apparently, the K1 clade was most likely a new clade, clustered with the HKU6 clade. Each sequence of K1 clade has $<89\%$ pairwise nucleotide sequence identity to the closest reference sequence (Bat coronavirus Mm_CoV-2 was found in Japan, accession number: LC469030.1) based on partial analysis of the RdRp. Furthermore, twenty-four sequences of this clade were diversely identified in the sites and were found in different bat

species, including *Myotis petax*, *Miniopterus schreibersii*, *Myotis macrodactylus* and *Rhinolophus ferrumequinum*, involving 14 sampling sites in Chungbuk, Gangwon and Jeju Island.

Ten betacoronaviruses were associated with *Rhinolophus ferrumequinum* only, which were divided into two groups (Figure 4). B17-155 and B18-50 were related to MERS-CoV from South Korea and MERS-related CoV from the United Arab Emirates identified in 2015, showing 85.1% sequence identity. The other eight sequences (B16-75, B17-60, B17-62, B18-82, B18-83, B18-92, B18-168 and B18-171) were grouped with the SARS-like CoV clade, sharing 99.6% sequence identity (B16-75, B17-60, B17-62, B18-82, B18-83, B18-92, B18-168 and B18-171) were grouped. In addition, our SARS-like CoVs only shared 86.7 to 87.0% nucleotide identity with SARS-CoV-2 and the bat coronavirus RaTG13 based on partial analysis of the RdRp.

4 | DISCUSSION AND CONCLUSION

This research sought to comprehensively investigate coronaviruses in bats at different geographical locations in South Korea from 2016 to 2019, to improve our understanding of bat species and bat CoVs strains, and to find relationships with bat coronavirus identified in other countries.

4.1 | Seasonal pattern of the bat coronaviruses in Korea

Previous studies indicated that the season affects the percentage of coronavirus positivity, with a higher coronavirus prevalence in the dry season in comparison with the wet season in Africa and Asia (Anthony et al., 2017). In addition, hibernating bats are a factor affecting the prevalence and diversity of coronavirus in the region during winter. Thus, it seems reasonable that during the awakening period (April), the average rate of coronavirus detection was higher than other months of the year, as observed in this study. By contrast,

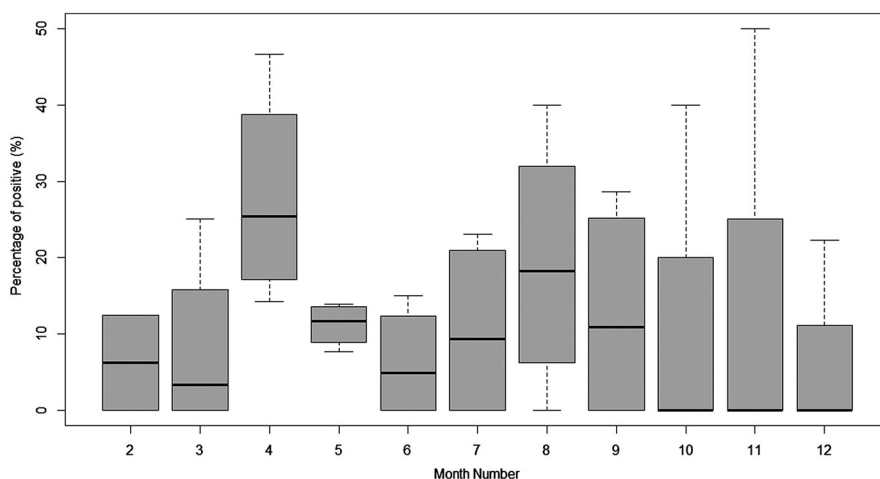


FIGURE 2 Box plot of the coronavirus positivity rate by month. Each box plot was pooled monthly for the percentage of coronavirus-positive samples from 2016 to 2019. Data are presented as median and interquartile range; minimum and maximum values are shown by whiskers

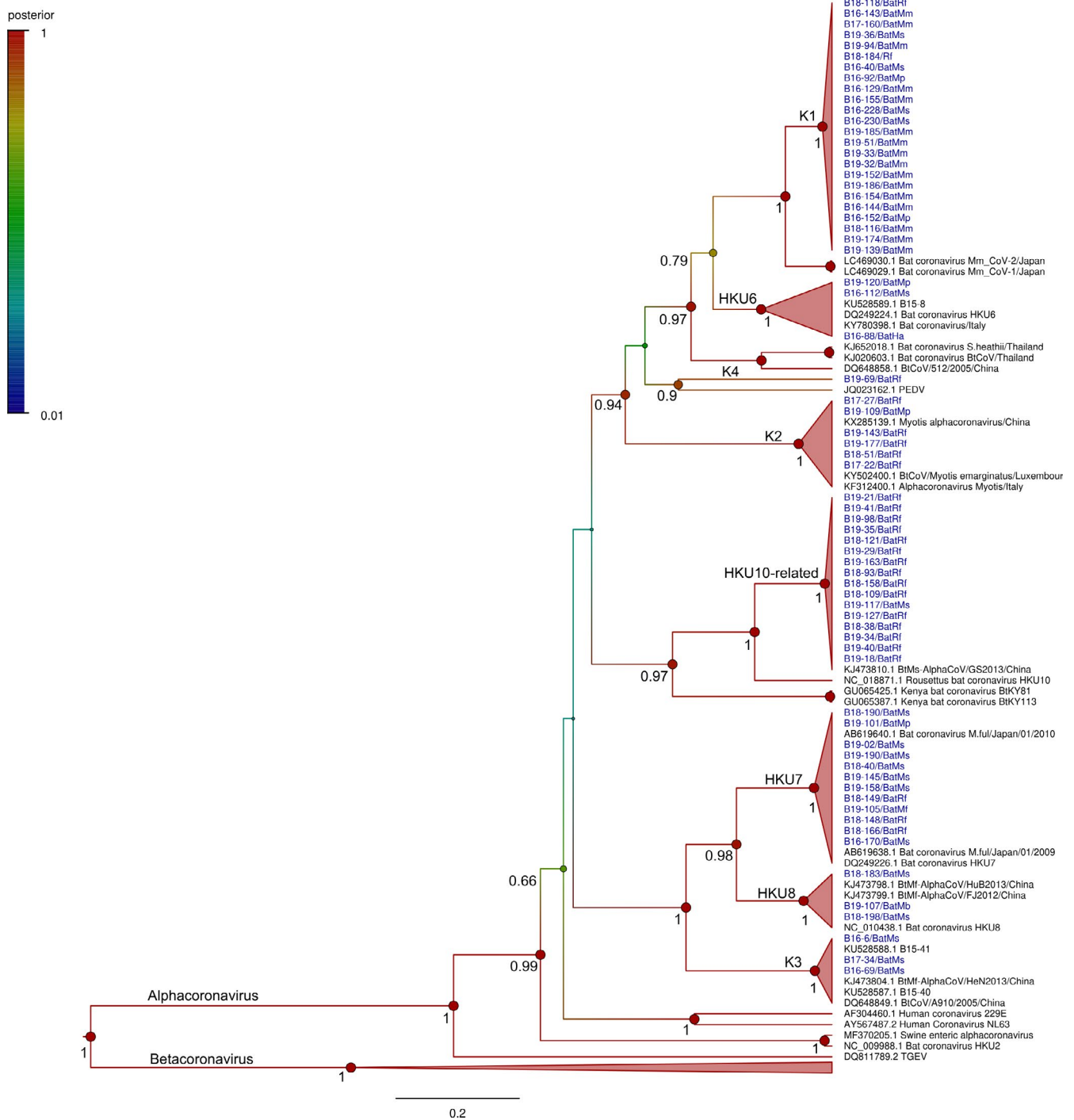


FIGURE 3 Bayesian phylogeny of 279 nucleotides from the alphacoronaviruses detected in this study with 30 reference sequences. Statistical support (posterior probability) of nodes is depicted using a gradual colour code of the tree, red indicating significant posterior probability values (> 0.95). For clarity of presentation, only posterior probability values above 0.65 are shown and values at crown positions were removed. Sequence names and main information is provided in taxa labels. Viruses detected in this study are coloured in blue

through the period of hibernation, the lower presence of coronavirus was observed. Especially in January, we did not find fresh faeces in the cave. Besides, the mean of positive rate was also high in August due to physical contact over the reproductive season. Thus, the physical contact and/or airborne transmission, and co-roosting of several bat species pre- and post-hibernation might lead to an increased viral transmission.

4.2 | Distribution and diversity

From the prevalence of coronavirus in the studied sampling sites, it was assumed that coronaviruses are circulating in Korean bats throughout the country. Betacoronaviruses were only detected in the north (Gangwon and Incheon) and the southwest

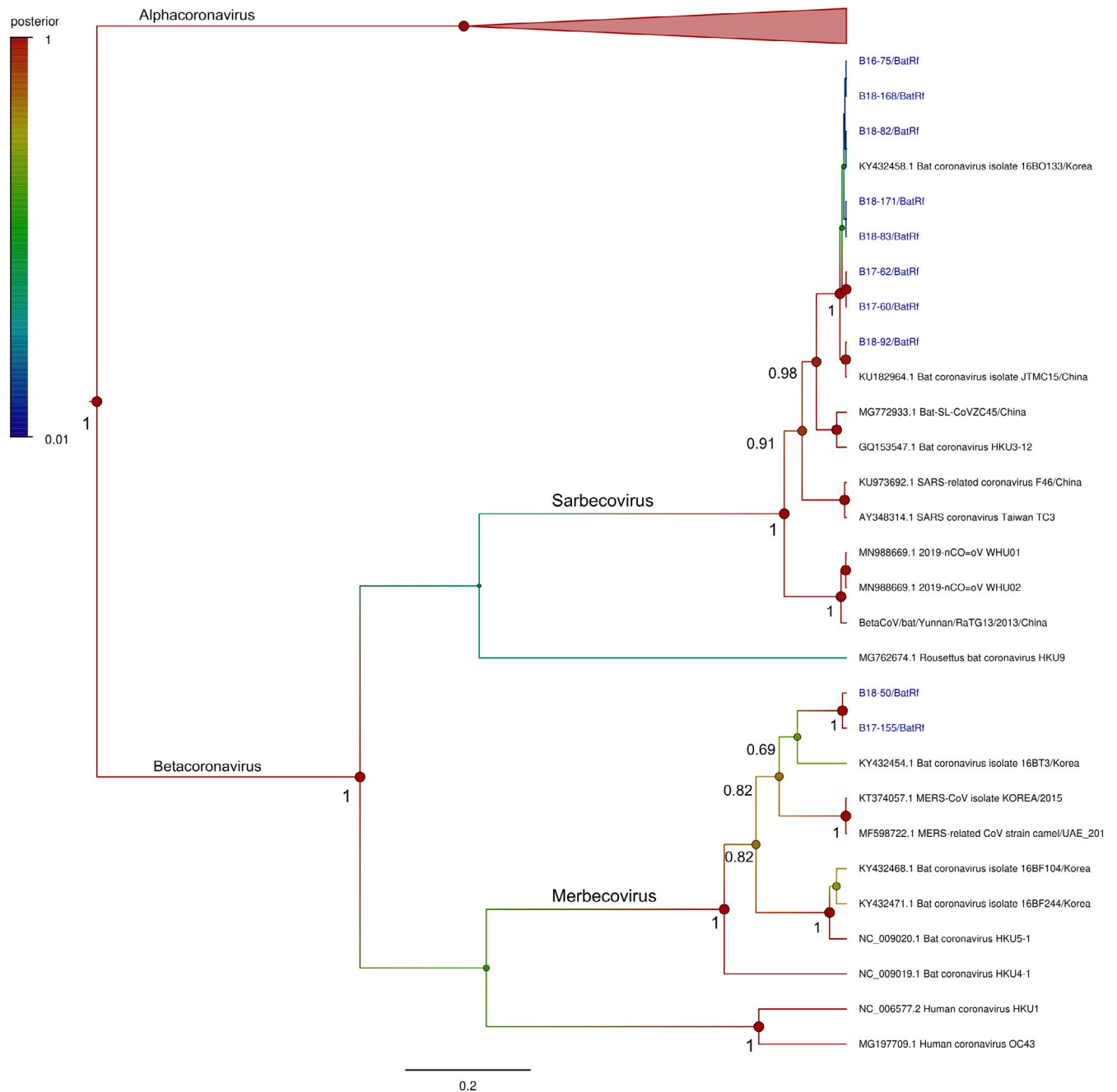


FIGURE 4 Bayesian phylogeny of 279 nucleotides from the betacoronaviruses detected in this study with 19 reference sequences. Statistical support (posterior probability) of nodes is depicted using a gradual colour code of the tree, red indicating significant posterior probability values (> 0.95). For clarity of presentation, only posterior probability values above 0.65 are shown and values at crown positions were removed. Sequence names and main information is provided in taxa labels. Viruses detected in this study are coloured in blue

(Gyeonggi and Jeonnam), whereas alphacoronaviruses were more widely detected at both mainland and island sampling sites (Figure 1), and were detected at higher rates than betacoronaviruses. In our study, the prevalence of alphacoronaviruses was approximately seven times higher. It is likely that bats are more susceptible to—and wider hosts of—alphacoronavirus than betacoronavirus (Anthony et al., 2017; Lazov et al., 2018; Lin et al., 2017; Wacharapluesadee et al., 2015). To date, seven of twenty-four Korean bat species were found to be associated

with alphacoronaviruses, and only four species were found to be associated with betacoronaviruses. Among these species, only *Rhinolophus ferrumequinum* was the major bat species from the site where both alphacoronaviruses and betacoronaviruses were found (Table 1).

Globally, the diversity and distribution of bat coronaviruses were indicated in association with biogeography and diversity of their host (Leopardi et al., 2018). This hypothesis probably accounts for heterogeneous circulation of alphacoronavirus.

It is especially important to explain the appearance and diversity of this putative new species of K1 clade (24 sequences) observed in four bat species (Table 2). Despite this, most of these alphacoronaviruses identified in Korean bats were close to bat coronavirus found in China in the same as well as different bat species.

4.3 | The potential of cross-species transmission and host switching

The mechanisms of cross-species transmission or spillover and host switching are still unclear. However, these mechanisms are considered the dominant evolutionary events involving recombination between different viruses, associated with the expansion of viral

host ranges or creation of a novel virus. It is widely accepted that cross-species transmission events have contributed to the genetic diversity and evolution of coronaviruses (e.g. SARS-CoV and HCoV) (Banerjee, Misra, Schountz, & Baker, 2018; Cui et al., 2019; Leopardi et al., 2018; Menachery, Graham, & Baric, 2017). Several different bat species crowding, co-roosting and co-hibernating may increase the potential for intraspecies and inter-species transmission of viral infections.

For instance, in the putative new K1 clade, three alphacoronaviruses (B19-36 in *Miniopterus schreibersii*, and B19-51 and B19-94 from *Myotis macrodactylus*) were detected in the same roost from different bat genera. In addition, B18-166 from *Rhinolophus ferrumequinum*, B19-101 from *Myotis petax*, and B19-145 and B19-158 from *Miniopterus schreibersii* belonging to HKU7 clade were also found in the same cave. In HKU8 clade, B18-198

TABLE 1 Information of the positive sample

No.	Clade/cluster	No. of positive	Bat species	Local detection
1	K1	24	<i>Miniopterus schreibersii</i>	Chungbuk
			<i>Myotis macrodactylus</i>	Gangwon
			<i>Myotis petax</i>	Jeju Island
			<i>Rhinolophus ferrumequinum</i>	
2	K2	6	<i>Myotis petax</i>	Chungbuk
			<i>Rhinolophus ferrumequinum</i>	Gangwon Gyeonggi
3	K3	3	<i>Miniopterus schreibersii</i>	Gyeongbuk Gyeongnam
4	K4	1	<i>Rhinolophus ferrumequinum</i>	Gyeongbuk
5	HKU6	3	<i>Hypsugo alaschanicus</i>	Chungbuk
			<i>Miniopterus schreibersii</i>	Gyeongbuk
			<i>Myotis petax</i>	
6	HKU7	12	<i>Miniopterus fuliginosus</i>	Gangwon
			<i>Miniopterus schreibersii</i>	Gyeongbuk
			<i>Myotis petax</i>	Gyeongnam
			<i>Rhinolophus ferrumequinum</i>	Jeju Island
7	HKU8	3	<i>Miniopterus schreibersii</i>	Gyeongbuk
			<i>Myotis bombinus</i>	Jeju Island
8	HKU10-related	16	<i>Miniopterus schreibersii</i>	Chungbuk
			<i>Rhinolophus ferrumequinum</i>	Chungnam Gangwon Gyeongbuk
				Incheon
				Jeju Island
				Jeonnam
9	SARS-like	8	<i>Rhinolophus ferrumequinum</i>	Gangwon
				Gyeonggi
				Incheon Jeonnam
10	MERS-related	2	<i>Rhinolophus ferrumequinum</i>	Gangwon

TABLE 2 Potential sharing of the certain bat coronaviruses among the major bat species of the sample collection sites. The bat species were confirmed by nucleotide sequence analysis of the *Cytochrome b* (*Cytb*) gene

Cases	Name	Date of collection	Sampling position	Latitude	Longitude	Bat species	Clade
1	B19-36/BatMs	4/11/2019	Gangwon	37.310306	128.528028	<i>Miniopterus schreibersii</i>	K1
	B19-51/BatMm	5/9/2019	Gangwon	37.310306	128.528028	<i>Myotis macrodactylus</i>	
	B19-94/BatMm	7/1/2019	Gangwon	37.310306	128.528028	<i>Myotis macrodactylus</i>	
2	B16-144/BatMm	7/19/2016	Gangwon	37.235667	128.355278	<i>Myotis macrodactylus</i>	K1
	B16-152/BatMp	7/19/2016	Gangwon	37.235667	128.355278	<i>Myotis petax</i>	
3	B19-105/BatMf	7/22/2019	Gyeongbuk	36.683083	128.20775	<i>Miniopterus fuliginosus</i>	HKU7
	B19-190/BatMs	9/25/2019	Gyeongbuk	36.683083	128.20775	<i>Miniopterus schreibersii</i>	
4	B18-166/BatRf	9/29/2018	Gangwon	37.397861	128.426528	<i>Rhinolophus ferrumequinum</i>	HKU7
	B19-101/BatMp	7/2/2019	Gangwon	37.397861	128.426528	<i>Myotis petax</i>	
	B19-145/BatMs	8/20/2019	Gangwon	37.397861	128.426528	<i>Miniopterus schreibersii</i>	
	B19-158/BatMs	8/24/2019	Gangwon	37.397861	128.426528	<i>Miniopterus schreibersii</i>	
5	B18-198/BatMs	12/25/2018	Gyeongbuk	36.683083	128.20775	<i>Miniopterus schreibersii</i>	HKU8
	B19-107/BatMb	7/22/2019	Gyeongbuk	36.683083	128.20775	<i>Myotis bombinus</i>	

and B19-107 were identified in *Miniopterus schreibersii* and *Myotis bombinus*, respectively, found in the same cave from different sampling year. Furthermore, the potential of host switching in family species may explain the detection of these alphacoronaviruses belonging to new K1 clade in *Myotis petax* and *Myotis macrodactylus* from the same (Table 2, case 2) or neighbouring roosting sites in Gangwon.

In our study regions, co-roosting behaviour was frequently observed in *Myotis* spp. and *Miniopterus schreibersii* with *Rhinolophus ferrumequinum*. Consequently, the frequency of interaction among these bat species provides conditions suitable for the spillover of a coronavirus. With respect to the frequent clustering of coronaviruses, the clusters associated with PEDV and BtCoV/512/2005 were most commonly contained *Murina*, *Myotis*, *Schotophilus* and *Pipistrellus* spp. (Leopardi et al., 2018). Thus, *Rhinolophus ferrumequinum* is likely to be a new host of these clusters as a result of cross-species transmission events, which were achieved by jumping from the bat genera *Myotis* or *Miniopterus* to *Rhinolophus*.

Overall, this study provided recent molecular epidemiology of bat coronaviruses in Korea through long-term and widespread surveillance. We also detected SARS-related and MERS-related CoVs that were closely related to bat coronaviruses identified in South Korea and China, and those betacoronaviruses detected in this study belonged to the major two clades, compared to the alphacoronaviruses (eight clades). The alphacoronaviruses were widely found in diverse bat species and were heterogeneous, which might be due to cross-species transmission events. While *Rhinolophus ferrumequinum* was the only species harbouring betacoronaviruses, alphacoronaviruses were observed to have considerable richness in terms of host species in Korea. However, this study has several limitations because only eleven of the

twenty-four bat species in Korea were included in this study, and direct collection of faecal samples from bats was not possible. There should be follow-up studies with direct sample collection from captured bats, and identification of the captured bat species based on genetic analysis.

These findings lead us to believe that further studies are needed to better understand the diversity, circulation and evolution of coronaviruses in bats. Certainly, the long-term surveillance of bat CoVs should be maintained and expanded to include more geographical locations and bat species.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The sequence data that support the findings of this study are openly available in Genbank (<https://www.ncbi.nlm.nih.gov/genbank/>) with the accession numbers MK991901 to MK991951.

ORCID

Sun-Woo Yoon  <https://orcid.org/0000-0003-2061-9743>

Dae Gwin Jeong  <https://orcid.org/0000-0002-0764-1404>

Hye Kwon Kim  <https://orcid.org/0000-0003-3458-3403>

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