VIRULENCE
2025, VOL. 16, NO. 1, 2502551
https://doi.org/10.1080/21505594.2025.2502551

Taylor & Francis
Taylor & Francis Group

VIRAL PATHOGENESIS

OPEN ACCESS Check for updates

Circulation characteristics of bat coronaviruses linked to bat ecological factors in Korea, 2021–2022

Min Chan Kim[a], Seong Sik Jang[a], Thi Van Lo[a], Ji Yeong Noh[a], Hyun A. Lim[a], Ha Yeon Kim[a], Da Young Mun[a], Kihyun Kim[b], Taek-Woo Lee[b], Yong Gun Choi[c], Sun-Woo Yoon[d], Dae Gwin Jeong[e,f], Sun-Sook Kim[b], and Hye Kwon Kim [a]

[a]Department of Biological Sciences and Biotechnology, College of Natural Sciences, Chungbuk National University, Cheongju, Republic of Korea; [b]Ecological Technology Research Team, Division of Ecological Application, National Institute of Ecology, Seocheon, Republic of Korea; [c]The Korean Institute of Biospeleology, Daejeon, Korea; [d]Department of Biological Sciences and Biotechnology, Andong National University, Andong, Republic of Korea; [e]Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea; [f]Bio-Analytical Science Division, Korea University of Science and Technology (UST), Daejeon, Korea

ABSTRACT

Considering that bat ecology alterations may be linked with pathogen spillover, research on bat coronaviruses, particularly on the infection and transmission pattern among bats in relation with their ecology, is essential. We captured bats distributed in Korea from 2021 to 2022, examined coronaviruses in oral swabs, feces, urine, and ectoparasites, and were able to detect alphacoronavirus. We investigated coronaviruses, but noted no substantial differences in the body condition index in the coronavirus-positive bats. Binary logistic regression analysis revealed that bat ecological factors that were significantly associated with coronavirus-positive were roost type, sample type, and bat species. Coronavirus-positive ectoparasite cases suggested additional study on the potential role of them as the viral transmission vectors or fomites. Reinfection of a different coronavirus in recaptured bats was evident, suggesting the possibility that coronavirus circulation can evade the potential protective immunity acquired from previous coronavirus infections. The present findings provide comprehensive information on the coronaviruses transmission dynamics within bat populations linked with bat ecology.

ARTICLE HISTORY
Received 12 August 2024
Revised 25 November 2024
Accepted 26 January 2025

KEYWORDS
Bat; ecology; coronavirus; transmission; diversity; ectoparasite; ; reinfection

Introduction

Bats are infected by diverse viruses, of which have been associated with the recent-emerging infectious diseases in human population. Direct evidence of zoonotic transmission between bats and human was identified as the cause of infectious diseases by Nipah and Hendra virus [1,2]. In addition, bat coronaviruses closely related to SARS-CoV, SARS-CoV-2, and MERS-CoV had been reported across the world, albeit there is no direct evidence of their zoonotic transmission between bats and human [3–5]. Thus, the zoonotic potential of bat-originated viruses has received attention in relation to potential pandemic.

Bat coronaviruses are one of the most common viruses reported in bat species. Most human coronaviruses are believed to share common ancestors with bats, which is the subject of ongoing research on the roles of bat coronaviruses in future potential pandemic [6]. Bats infected with bat coronaviruses do not seem to displays severe clinical symptoms. Experimental infection of bat coronaviruses did not display any clinical signs in *Leschenault rousette* bats (*Rousettus leschenaulti*) [7]. SARS-CoV-2 infection in Brazilian Free-Tailed Bats (*Tadarida brasiliensis*) and big brown bat (*Eptesicus fuscus*) did not develop any clinical disease as well [8,9]. However, although we could not confirm the endemic or epidemic status of bat coronavirus-associated infectious diseases in the studied bat populations, the viral transmission could be maintained among them, exhibiting seasonality and cross-species sharing [10].

Considering that viruses are obligate parasite requiring hosts for their replication, viruses can evolve inside the hosts. In this regard, it would help to analyze the correlation between hosts factors of bats and bat viruses. Recent studies have revealed that bat ecology can be affected by human activities, which might increase the zoonotic risk of bat viruses. Human impact such as through agriculture, deforestation, and mining has contributed to the high bat coronavirus prevalence

CONTACT Hye Kwon Kim khk1329@chungbuk.ac.kr; Sun-Sook Kim sskim108@nie.re.kr
Supplemental data for this article can be accessed online at https://doi.org/10.1080/21505594.2025.2502551

[11]. In addition, habitat loss of bats due to human land use has been associated with changes in bat ecology, which has increased the spillover risk [12,13]. In addition to the human factors, diverse ecological, behavioral, and molecular aspects of bats can be considered to evaluate why several potential zoonotic viruses have been circulating in bat species [14]. Prediction model integrating data on host ecology has been shown to make more powerful prediction for zoonotic reservoirs [15].

Thus, efforts have been made to elucidate the bat ecological conditions that affect the circulation of bat viruses among the bat populations. For instance, food shortage and habitat changes were associated with winter pulse of Hendravirus excretion, and alpha- and betacoronaviruses were highly circulating in juvenile and immature bats [16,17]. However, there exists knowledge gaps regarding infection dynamic of bat coronaviruses within bat species [6]. Therefore, in this study, several natural bat habitats were selected and chronologically monitored for bat coronaviruses as well as ecological factors of the habituated bats so as to study their relationship and the potential risk variables for bat coronavirus circulations in a specific population.

Materials and methods

Samples & data collection

From January 2021 to December 2022, a total of 15 species of bats were captured in their natural habitats across 7 provinces of South Korea (Supplementary Figure S1, A). Using mist nets, we captured bats at cave and abandoned mine entrances during the active season. Inside-cave and abandoned mine captures in the winter season were conducted using only hand nets, without using any specialized equipment. Bat capturing began 2 h before the sunrise, when the bats returned to their roosts after feeding in the morning. Our sampling time was adjusted based on the sunrise times for each sampling day. Once captured, the bats were individually placed into cotton bags (20 × 14 cm; Ecotone, Poland). After completing 1 h of bat capturing, we proceeded with urine and guano sampling, oral swabs, banding, and measuring body weight (in grams) and forearm length (in millimeters). After capturing the bats, they were marked with individually numbered metal rings (Incoloy Ring, PORZANA, diameter 3.5 mm, 2.8 mm, and 2.4 mm) attached to their forearm before releasing them. We identified bat species based on the criteria described by Yoon et al. [18], NIBR [19], and Jo et al. [20]. Age discrimination was conducted during two periods, from late July to mid-September, when juvenile and adult bats can be reliably distinguished, following the criteria outlined by Gannon [21]. To elaborate further, bats collected using mist nets were acclimated in cotton bags for 1 hour. If urine droplets were observed when the bat was removed from the bag, the droplets were collected using sterile swabs or directly into viral transport media. For guano samples, feces deposited in the cotton bags during the acclimation period were collected. For oral samples, we used the NFS-1 swab applicator (Noble Bio, Korea). Just before handling each bat, a sterile swab was removed from its packaging, and the bat's oral cavity was gently swabbed to collect saliva samples. If ectoparasites were found on the bat, they were collected using sterilized forceps. All samples were stored in viral transport media (Noble Bio, Korea), placed in insulated coolers with ice packs, and transported immediately to the laboratory. Upon arrival, samples were stored at −80°C until further analysis. When the samples were collected, the date, location, bat species, sex, colony size, roost type, and body condition index (BCI) were recorded. The roost types were categorized according to the following criteria. The study site has been continuously monitored for the past 20 years, and the species composition and population size within the cave have remained stable. Based on this extensive data, we divided the roost types into five distinct periods, each reflecting both seasonal and reproductive phases of the bat species present. These periods include early hibernation (December 1 to January 31), end of hibernation (February 1 to March 20), diurnal roosting (excluding maternity and hibernation periods), pre-natal period (May 1 to June 30), and post-natal period (July 1 to August 20). The BCI was calculated as a ratio of mass and forearm length (mass/forearm length). In addition, the air samples were collected using an MD8 airport (Sartorius, Germany) at the entrance of the cave (Supplementary Figure 1, B). Air collectors were strategically installed at key points within the cave where all resident bats pass through, particularly in the main passageways. The cross-sectional area of these movement corridors was approximately 2–3 meters in width and 4–5 meters in height. The collectors operated at a flow rate of 30 liters per minute for 40 min, and to maximize sample collection, the filters were replaced twice at the same location, resulting in a total air volume of approximately 1,200 liters per session.

Notably, three natural habitats of a bat species, *R. ferrumequinum* were selected for the chronological monitoring of bat coronaviruses in the bat species: Sanpyeong mine in Anseong (February–April and

December 2021), Myodong mine in Hampyeong (January, March, April, August, October, December 2021), and Beollari cave in Jeju (January, March, June, August, and December 2021). Sampling was conducted outside the birthing period (June to late July) for bat species inhabiting the cave in our study area. Specifically, surveys were carried out at the end of May, prior to the breeding season, and at the end of July, after birthing had already occurred. To minimize potential impacts on reproductively active females and their offspring, we avoided sampling during the peak birthing period. This study was approved by the Research Planning Review Committee of the National Institute of Ecology (NIEIACUC-2021-001). We declare that we adhered to the Wildlife Protection and Management Act of Korea as well as the Institutional Research Ethics Regulations and Guidelines. All handling and sampling permissions were obtained from the seven corresponding local governments on each sampling year. We also have adhered to ARRIVE guideline which checklist is also uploaded.

RNA extraction

All samples, except for the ectoparasite and air samples, were vortexed and centrifuged at 3,000 x*g* for 15 min at 4°C. The ectoparasites were mixed with 1 mL of viral transport media and homogenized with a 2.8-mm ceramic bead in 1.5 tube (Crio Manufacturing Corporation, USA) using a bead homogenizer, while the filters from air samplings were melted with 10 mL of viral transport media in a petri dish (SPL, Korea). The prepared samples were centrifuged at 3,000 x*g* for 15 min at 4°C. Then, all supernatant (250 uL) was used for RNA extraction using Trizol LS (Ambio, USA), following the manufacturer's manual. The final RNA was eluted into 30 uL of DEPC water.

Consensus primers-based RT-nested PCR for coronaviruses screening

The cDNA was synthesized using the M-MLV reverse transcriptase kit (Promega, USA) from the extracted RNA as per the manufacturer's instructions. To detect the coronaviruses, consensus primers-based nested PCR targeting the conserved RdRp region was performed. In the first PCR, a mixture containing 2 µL of cDNA, PCR Master Mix (Bioneer, Korea), 1 µM of each primer, and nuclease-free water (NFW) was used to a final volume of 20 µL. The primers used included Chu-RdRp-N1-F (5'-GGKTGGGAYTAYCCKAARTG-3') and Chu-RdRp-N1-R (5'-TGYTGTSWRCARAAYTCRTG-3'). In the second PCR, a mixture containing 1 µL of the first PCR product, PCR Master Mix, 1 µM of each primer, and NFW was used. The primers used in this study included Chu-RdRp-N2-F (5'-GGTTGGGACTATCCTAAGTGTGA-3') and Chu-RdRp-N2-R (5'-CCATCATCAGATAGAATCATCAT-3'). Both PCRs were conducted under the same conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 48°C for 30 s, and 72°C for 40 s, and a final extension step of 72°C for 5 min. The PCR products were visualized on a 1% agarose gel to observe the target band. If the target band was observed, it was purified by the gel extraction method using the GeneAll® ExpinTM Combo GP kit (GeneAll, Korea) as per the manufacturer's protocol. Finally, the purified PCR products were dispatched to Cosmogenetech Company (Seoul, Korea) for sequencing. The obtained sequences were aligned using BioEdit (version 7.2) software, and the target sequence was confirmed by BLAST search (NCBI, USA).

Identification of ectoparasite species collected from the bats

DNA was extracted from the ectoparasite samples, from which coronavirus was detected using the QIAamp DNA Mini kit (QIAGEN, Germany) according to the manufacturer's instructions. To identify the parasite species, PCR specific for the parasite's 16S rDNA was performed [22]. PCR was performed with a total volume of 20 uL using 2 uL of template DNA, 10 uL of PCR Master Mix (Bioneer, Korea), a volume of 1 uM primers each and volume of NFW. The primers used in this study were Tick 16S rDNA-F (5′-TTGGGCAAGAAGACCCTATGAA-3′) and Tick 16S rDNA-R (5′- CCGGTCTGAACTCAGATCAAGT-3′). The conditions for PCR are as follows; 95°C for 5 min, followed by 40 cycles of 95°C for 45 s, 55°C for 1 min, and 72°C for 90 s with a final extraction at 72°C for 1 min. The PCR products were purified by observing the electrophoresis target band using 1% agarose gel and then processed with gel-extraction using the GeneAll® ExpinTM Combo GP (GeneAll, Korea) protocol. The PCR products were submitted to cosmogenetech company (Seoul, Korea) for sequencing with both the primers. The obtained sequence was aligned through BioEdit (7.2ver) software, and the target sequence was analyzed with BLAST search.

Phylogenetic analysis

A total of 66 partial RdRp sequences of the coronaviruses were deposited at the GenBank (OQ401100 to OQ401158 and OOR700183 to GOR700189). The partial RDRP sequences of coronaviruses in this study were aligned with the reference sequences using

ClustalW implemented in BioEdit version 7.2 [23,24]. Each sequence was trimmed based on the portions that had 100% length matching with the reference. Phylogenetic analysis was performed using the Tamura-Nei model in MEGA 11 software, and a phylogenetic tree was constructed with maximum likelihood, 2,000 bootstrap replicates. The resulting tree was saved in the Newick (nwk) file format and was further edited using Treeviewer software [25].

Statistical analysis

Each sample type (e.g. oral swabs, urine, guano, and ectoparasites) was analyzed separately, with positivity assessed independently for each sample type per bat, ensuring that the detection ratio reflected the positivity rate for each sample type across all individuals without inflating the ratio due to multiple positive samples from the same bat. Student's *T*-test was used for the comparison of numeric data; for instance, BCI data. The statistical analysis and graphs generation were performed using GraphPad software (Ver. 9.5.0). Furthermore, binary logistic regression analysis was performed using SPSS software (Ver. 26) to examine the correlation between coronavirus positivity as a dependent variable and bat ecological factors such as the year, quarter, location, total number of bats, number of species in habitat, number of bats per one colony, sample type, roost type, species, sex, BCI, and others as independent variables (Supplementary Table S1). The date was divided into quarters. BCI data was considered as scale variables, while the year, quarter, location, total number of bats, number of species in habitat, number of bats per one colony, sample type, roost type, species, and sex treated as nominal variables. Logistic regression analysis was performed with bootstrap 1000.

Results

Coronavirus detection and distribution

A total of 1,530 bat-associated samples in 934 bats were collected, which included oral swabs (501), guano (528), urine (207), and ectoparasite samples (294), along with biological and ecological information such as the sex, age, body weight, and forearm length. Moreover, 13 air samples were collected. When 1,543 samples were tested for the presence of coronavirus through RT-nested PCR targeting the RNA-dependent RNA polymerase (RdRp) gene and sequencing analysis, Alphacoronaviruses were detected in 4.86% (75/1,543) of samples, which were collected from bat habitats in 6 out of 7 regions in Korea.

When examining the monthly count of samples for detecting the coronavirus and their positivity rates, a higher number of samples tested positive for the coronavirus isolated during the bat's breeding season, spanning June to August. Regarding the positivity rates, the variations in sample sizes for each month should be considered, as it could give relatively higher values even when the actual count of samples with coronavirus detection was lower (Figure 1a).

Among the 15 bat species investigated, 7 tested positive, which included *R. ferrumequinum* (21/75), *Miniopterus fuliginosus* (12/75), *Myotis macrodactylus* (26/75), *Myotis aurascens* (3/75), *Murina hilgendorfi* (4/75), *Myotis petax* (5/75), and *Myotis bombinus* (4/75). All samples obtained through air collecting were identified to be negative for coronaviruses.

On examining the positive rates of coronavirus based on regions and species, Baram Cave in Pyeongchang (9.09%; 5/55), Sut Cave in Mungyeong (4.87%; 15/308), Sanpyeong Mine in Anseong (5.78%; 10/173), Beollari Cave and Buk Oreum Cave in Jeju (4.09%; 27/660), Myodong mine and Deogyang Mine in Hampyeong (2.13%; 5/235), and Gossi Cave in Yeongwol (11.82%; 13/110) had the highest rates, in a descending order (Figure 1b). When considering the species, the highest positive rates were detected in *Murina hilgendorfi* (21.05%; 4/19), *Myotis petax* (20.00%; 5/25), *Myotis macrodactylus* (12.50%; 26/207), *Myotis aurascens* (5.66%; 3/53), *R. ferrumequinum* (3.76%; 21/559), *Miniopterus fuliginosus* (3.11%; 12/386), and *Myotis bombinus* (2.11%; 4/190), in a descending order (Figure 1c).

Alphacoronavirus diversity in Korean bat species

Based on the nucleotide sequences of partial *RdRp*, phylogenetic analysis and BLAST results indicate that all detected coronaviruses belong to the Alphacoronavirus (4.68%, 75/1,543) group.

A phylogenetic tree was constructed using alignments between the sequences from positive samples and reference sequences, with a focus on a 212-bp region of matching bases. The sequences of length <212 bp, detected in positive samples, were excluded from the tree-building process. As a result, the tree was constructed using sequences from 66 to 75 positive samples.

Among the Alphacoronaviruses discovered in this study, six clades were identified: B1, B2, Myosis/China, *M. ful*/Japan, B15–41/Korea, and B15–40/Korea (Figure 2).

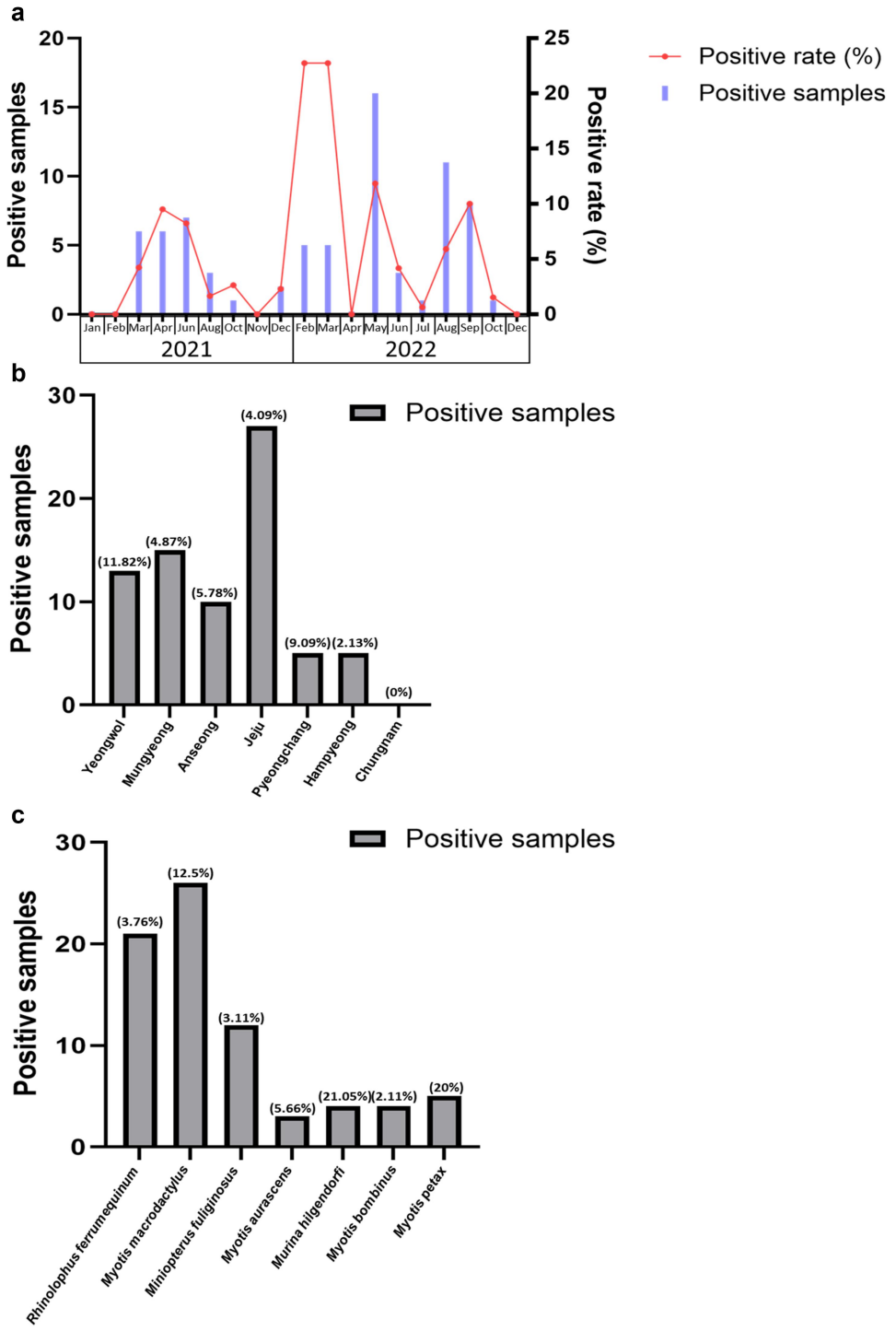

Figure 1. The graph depicting the number and ratio of samples in which the coronavirus was detected. (a) A graph depicting the number and ratio of samples wherein the coronavirus was detected, categorized by the months of sample collection during 2021–2022. The red dots represent the positivity rate, while the blue bars indicate the number of coronavirus-positive samples. (b) Graph showing the number of samples detected positive for coronavirus by region. Numbers in parentheses indicate percentages. (c) Graph showing the number of samples detected positive for coronavirus by bat species. The numbers in parentheses represent percentages.

The B1 clade was mainly composed of positive samples related to *Myotis macrodactylus* from Jeju and Yeongwol, with a reference sequence of Bat coronavirus Mm_CoV-2 (Genbank accession number, LC469030.1) discovered in Japan. The B2 clade was primarily composed of positive samples related to *R. ferrumequinum* from five regions excluding Yeongwol, as well as positive samples related to three other species. The B2 clade

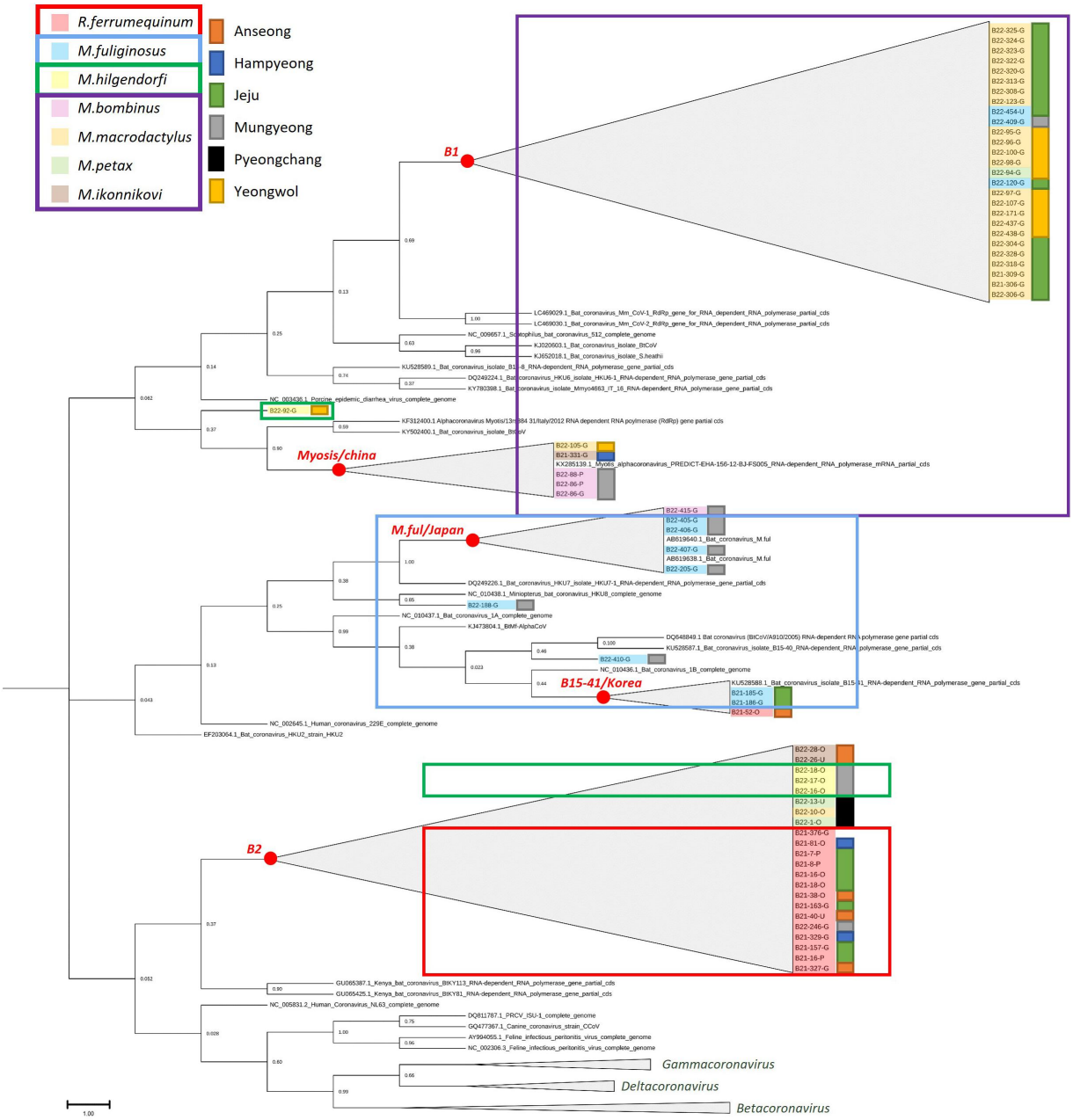

Figure 2. A phylogenetic tree representing the maximum likelihood of nucleotide sequences for 59 alpha-coronavirus sequences detected in the study and 55 reference sequences. The reference sequences of coronaviruses, other than Alphacoronavirus reference sequence, were grouped into respective clades according to their taxonomic classification. The red circles below the graph represent the clades, and the red text indicates the clade name. The detected viruses in this study are represented by boxes, with background colors indicating the related species and colors indicating the geographic location of the collected samples.

appeared to be most closely related to Kenya bat coronavirus BtKY81 (GenBank accession number, GU065425.1).

There are also viruses that form clade (*M. ful*/Japan clade) along with references that were all previously reported Alphacoronaviruses from East Asian countries, including South Korea, China, and Japan. Alphacoronaviruses in the clade were all samples collected from Mungyeong, and 4 out of 5 positive sequences matched with bat species *M. fuliginosus*. The alphacoronaviruses in Myosis/China clade consisted of three regions and three species, while B15–41 clade was clustered with alphacoronavirus found in Korea, 2015. The alphacoronaviruses, 22B–92-G and 22B–188-G which did not belong to any clade with another alphacoronaviruses in this study were found to be closely related to porcine epidemic diarrhea virus (PEDV) and Miniopterus bat coronavirus HKU8.

Alphacoronaviruses from *R. ferrumequinum* were mostly detected in the B2 clade, while those from *Miniopterus fuliginosus* were detected in *M. ful*/Japan and B15–41 clades. Alphacoronaviruses from *Myotis sp.* were in clades B1 and Myosis/China. This bat species-specific alphacoronaviruses were not detected in any specific region of the bat habitats, instead they were distributed across different regions.

Identification of coronaviruses in ectoparasites collected from the captured bats

In this study, 294 ectoparasites were collected from the captured bats. The parasitic sample that tested positive revealed two types of extracellular parasites upon morphological classification (Figure 3a,b). Among them, coronaviruses were detected in six ectoparasites. Based on the 16S rDNA sequence analysis [22], the ectoparasites, B21–7-P, B21–8-P, B21–16-P, and B21–17-P belonged to *Megastrebla*, while B22–86-P and B22–88-P belonged to *Spinturnix* (Table 1). This result suggests that the 16S rDNA sequence analysis is consistent with morphological classification. Among the coronavirus-positive ectoparasites, in two cases, the coronavirus was positive in the captured bats from which the ectoparasites were collected. Comparison of the partial RDRP sequences from ectoparasites and bats of their origin (Supplementary Figure S2, Supplementary Table S2) revealed sequence identities of 99.6% in the samples B21–16 (Oral) and B21–16-P (*Megastrebla*) (Figure 3c) and 100% for B22–86 (Guano) and B22–86-P (*Spinturnix*), respectively (Figure 3d).

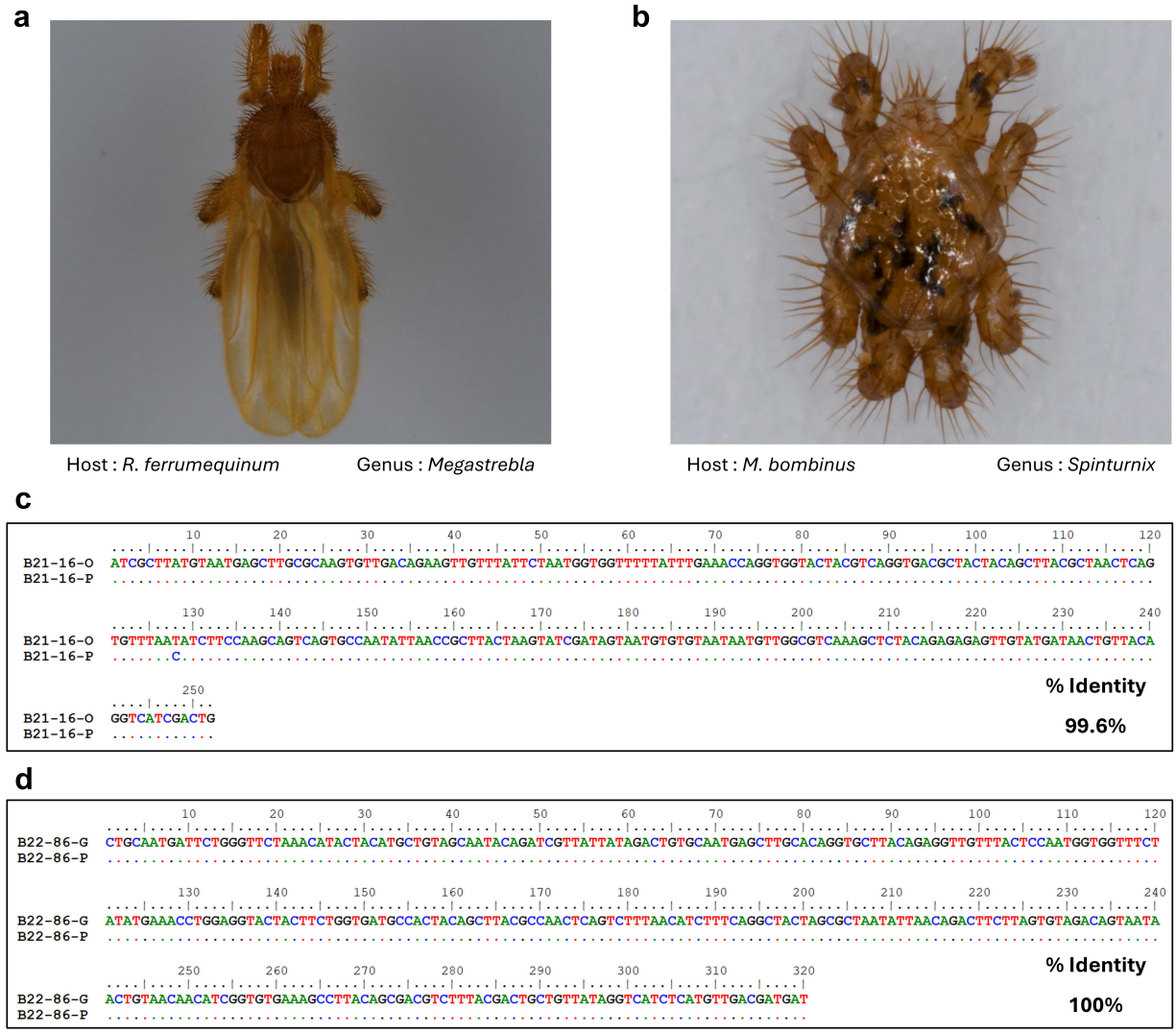

Figure 3. External parasites collected from bats and a sequence comparison between the coronavirus detected in samples obtained from bats and the coronavirus detected in the external parasites attached to the bats at the time of sample collection. (a) Collected bat ectoparasite in *R. ferrumequinum*, estimated to belong to the genus *Megastrebla*. (b) Collected bat ectoparasite in *M.Bombinus*, estimated to belong to the genus *Spinturnix*. (c) Sequence comparison between coronavirus detected in oral swab samples collected from ID: E001389 *(R. ferrumequinum)* and coronavirus detected in the external parasite. (d) Sequence comparison between coronavirus detected in guano samples collected from ID: I001876 (*M. bombinus*) and coronavirus detected in the external parasite.

Table 1. Ectoparasite species identification BLAST results; the top three results obtained were through a BLAST analysis using the ectoparasite sequences obtained from each ectoparasite sample.

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	*Per. ident	**Acc. Len	Accession number
21B–7-T	*Megastrebla nigriceps* "1" Di138 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla nigriceps "1" Di138*	383	383	100%	1.00E–101	95.78	494	DQ133049.1
	Megastrebla parvior parvior 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla parvior parvior*	351	351	99%	3.00E–92	93.64	490	DQ133024.1
	Megastrebla nigriceps "2" Di139 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla nigriceps "2" Di139*	318	318	99%	3.00E–82	91.1	489	DQ133032.1
21B–8-T	*Megastrebla nigriceps* "1" Di138 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla nigriceps "1" Di138*	383	383	100%	1.00E–101	95.78	494	DQ133049.1
	Megastrebla parvior parvior 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla parvior parvior*	350	350	99%	1.00E–91	93.62	490	DQ133024.1
	Megastrebla nigriceps "2" Di139 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla nigriceps "2" Di139*	320	320	100%	8.00E–83	91.14	489	DQ133032.1
21B–16-T	*Megastrebla nigriceps* "1" Di138 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla nigriceps "1" Di138*	390	390	99%	6.00E–104	95.85	494	DQ133049.1
	Megastrebla parvior parvior 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla parvior parvior*	355	355	99%	2.00E–93	93.36	490	DQ133024.1
	Megastrebla nigriceps "2" Di139 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla nigriceps "2" Di139*	326	326	99%	2.00E–84	91.25	489	DQ133032.1
21B–17-T	*Megastrebla nigriceps* "1" Di138 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla nigriceps "1" Di138*	383	383	100%	1.00E–101	95.78	494	DQ133049.1
	Megastrebla parvior parvior 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla parvior parvior*	351	351	99%	3.00E–92	93.64	490	DQ133024.1
	Megastrebla nigriceps "2" Di139 16S ribosomal RNA gene, partial sequence; mitochondrial	*Megastrebla nigriceps "2" Di139*	318	318	99%	3.00E–82	91.1	489	DQ133032.1
22B–86-T	*Spinturnix myoti* isolate H3 16S ribosomal RNA gene, partial sequence; mitochondrial	*Spinturnix myoti*	278	278	100%	3.00E–70	99.35	369	EU784885.1
	Spinturnix myoti isolate e12 16S ribosomal RNA gene, partial sequence; mitochondrial	*Spinturnix myoti*	272	272	100%	1.00E–68	98.69	370	EU784873.1
	Spinturnix myoti isolate ec1 16S ribosomal RNA gene, partial sequence; mitochondrial	*Spinturnix myoti*	272	272	100%	1.00E–68	98.69	386	FJ225969.1
22B–88-T	*Spinturnix myoti* isolate H3 16S ribosomal RNA gene, partial sequence; mitochondrial	*Spinturnix myoti*	278	278	99%	3.00E–70	99.35	369	EU784885.1
	Spinturnix andegavinus isolate H1 16S ribosomal RNA gene, partial sequence; mitochondrial	*Spinturnix andegavinus*	274	274	100%	4.00E–69	98.7	369	EU784873.1
	Spinturnix myoti isolate e12 16S ribosomal RNA gene, partial sequence; mitochondrial	*Spinturnix myoti*	272	272	99%	1.00E–68	98.69	370	FJ225969.1

*Per. Ident: (Percent Identity) the highest percent identity for a set of aligned segments to the same subject sequence.
**Acc. Len: (Accession Length) the number of nucleotides or amino acids in the result sequence identified by the accession number 3.

Coronavirus detection of recaptured bat cases

During the sample collection from the captured bats, five cases of recaptured bat samples were recorded (bat IDs: E000318, E001259, E001257, E001270, and A002616) in same roost site, wherein the coronavirus was detected more than once (Supplementary Table 3). In the cases of E000138 and E001259, coronaviruses were not detected in March and February of 2021, respectively, rather was detected in October and December of 2021. In the case of E001257 and A002616, the coronavirus was detected on February 2021 and June 2022, respectively, but not in March 2021 and July of 2022. Notably, in the case of E001270, the coronavirus was detected in both March and December of 2021 (Figure 4a). The coronaviruses detected in a bat E001270 (*R. ferrumquinum*) shared 72.3% sequence identity in partial RDRP region (Figure 4b), belonging to different clades of B15–41/Korea and B2, respectively (Figure 2).

Relationship between BCI of the captured bats and coronavirus-positive results

The comparison results of the BCI (the body mass divided by forearm length) between coronavirus-positive and -negative samples for each month from 2021 to 2022, except for the difference in August, indicated minimal variation. Statistical analysis was conducted on species (*M. fuliginosus, R. ferrumequinum, M. aurascens, M. macrodactylus*) showing sufficient positive and negative sample data for BCI analysis. Particularly, statistically significant differences were detected in three species (i.e. *R. ferrumequinum*; P-value 0.004, *M. fuliginosus*; P-value 0.022, *M. macrodactylus*; P-value 0.028). *M. fuliginosus* and *M. macrodactylus*, the bats that tested positive for coronavirus, exhibited significantly lower BCI when compared to negative bats. However, for *R. ferrumequinum*, the opposite trend was observed (Figure 5).

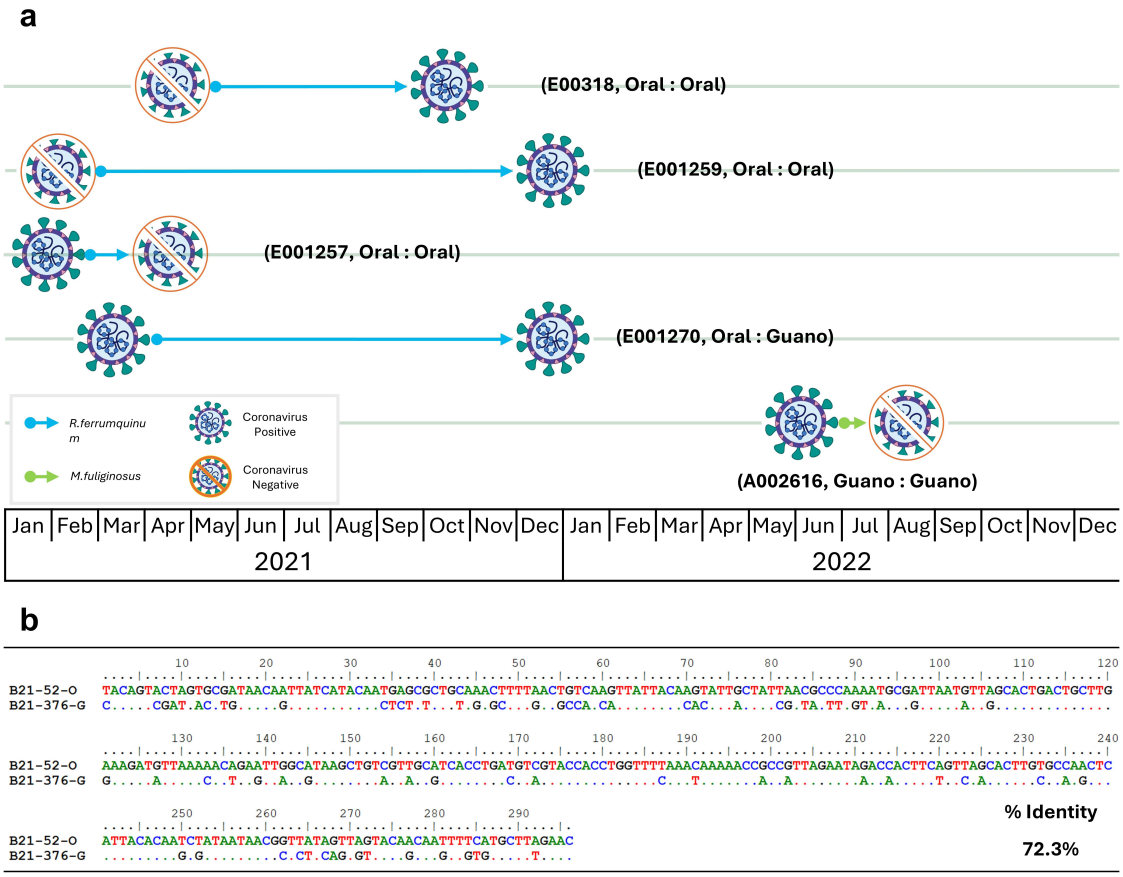

Figure 4. (a). Representation of the recaptured bats, arranged chronologically based on whether they were infected with the coronavirus at any point. Blue arrows indicate samples collected from *R. ferrumquinum*, while the green arrows represent samples collected from *M. fuliginosus*. For ID: E001270, coronavirus was detected both before and after recapturing. For ID: E00318 and E001259, detection occurred only after recapturing. On the other hand, for ID: E001257 and A002616, coronavirus was detected only before recapturing. (b). The picture comparing the sequences of the coronavirus detected before and after recapturing in ID: E001270 (*R. ferrumequinum*).

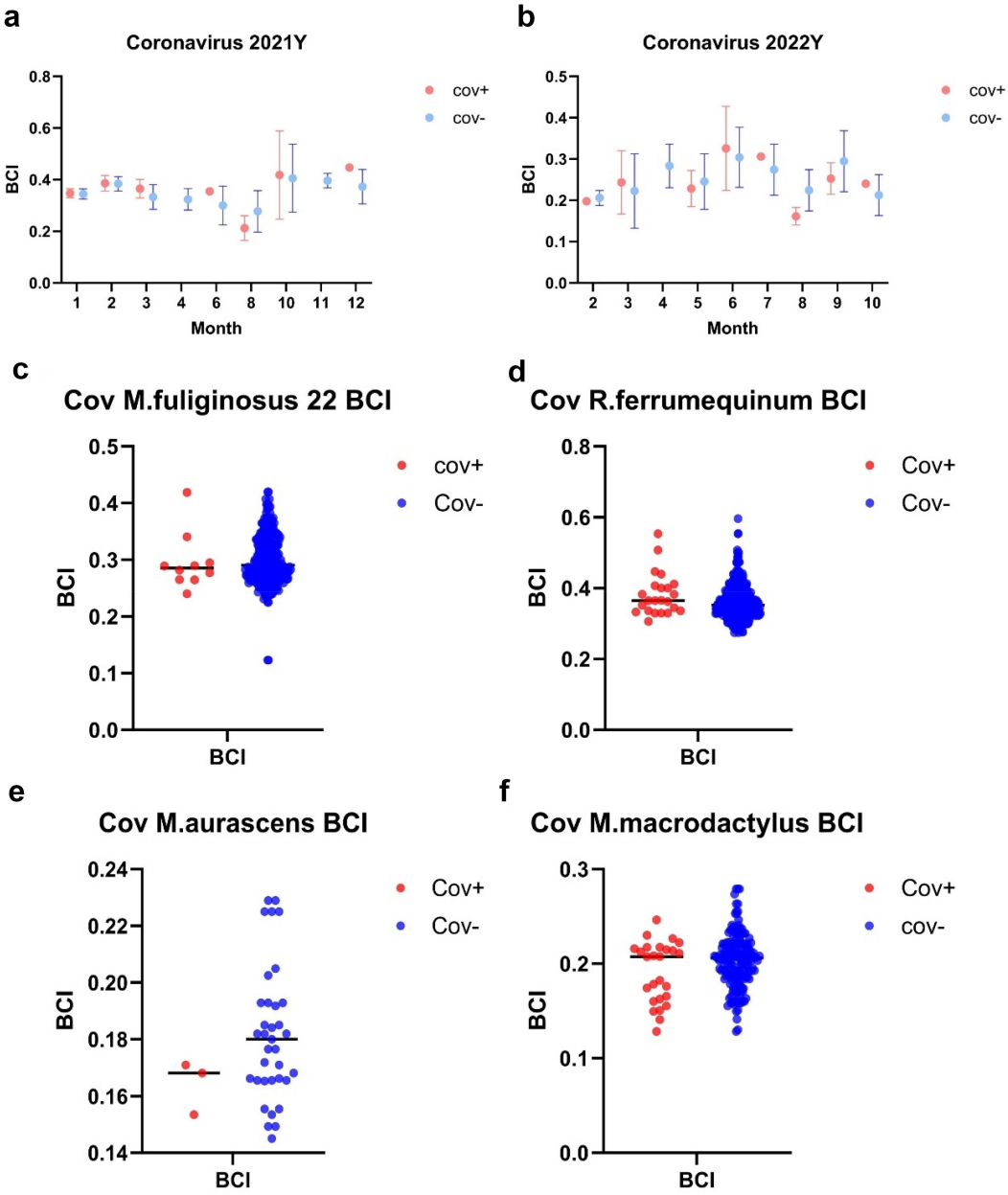

Figure 5. A graph comparing the positive and negative data of coronaviruses in bat-related samples with the body condition index (BCI) data of the bats. (a-b) a graph comparing BCI by month for each year. (c-f) a graph comparing BCI by species differences by bat species.

Binary logistic regression analysis of bat-related factors and coronavirus infection

Binary logistic regression analysis was conducted using bat-related data and information on the presence of coronavirus infection on a total of 1,530 samples (excluding air collection samples and samples for which statistical analysis was not feasible). Among these, 129 samples had missing data and were not included in the analysis, leaving a total of 1401 samples for analysis. Owing to the substantial missing values in the "age" data, this variable was excluded from the logistic regression analysis.

Review of these results indicated that the P-values for the sample type, roost type, and species were significant within a 95% confidence interval, implying their relevance in the analysis. Conversely, other variables were found to be statistically insignificant. Upon analyzing the outcomes related to the "roost type," which reflects the condition of bats at the time of sample collection, a pattern emerged wherein samples collected during "diurnal roost" were associated with a decreased likelihood of coronavirus detection when compared to those collected during the "post-natal" period. As for the "sample type" variable, statistically significant distinctions were recorded between

Table 2. Binary logistic regression analysis between coronavirus positives and independent variables of bat ecology.

		Beta	Standard Error	P-value	Oddss ratio (OR)	95% Confidence interval of OR
*Year	2021	0.682	0.557	0.221	1.977	0.664 – 5.890
**Quarter	Q4			0.297		
	Q1	−4.311	3.292	0.190	0.013	0.000 – 8.502
	Q2	22.124	3161.406	0.994	4057209104.224	0.000 – 0.000
	Q3	−2.032	1.499	0.175	0.131	0.007 – 2.472
***Location	Yeongwol			0.298		
	Mungyeong	11.543	3743.334	0.998	103057.263	0.000 – 0.000
	Hampyeong	12.689	3743.334	0.997	324125.216	0.000 – 0.000
	Jeju	13.805	3743.333	0.997	989724.382	0.000 – 0.000
	Anseong	8.512	3743.334	0.998	4975.927	0.000 – 0.000
	Pyeongchang	−3.751	3.380	0.267	0.023	0.000 – 17.709
****Total No. of Bat	1000≤x < 3000			0.768		
	1≤x < 100	−1.926	4.389	0.661	0.146	0.000 – 793.895
	100≤x < 500	13.457	3743.335	0.997	698694.987	0.000 – 0.000
	500≤x < 1000	1.284	3.298	0.697	3.612	0.006 – 2317.002
*****No. of Species	5<x ≤ 10			0.610		
	x ≤ 2	−2.196	6.284	0.727	0.111	0.000 – 24818.377
	2<x ≤ 5	−2.437	3.240	0.452	0.087	0.000 – 50.086
******No. of bats per one colony	1000≤x < 3000			0.756		
	1≤x < 100	−16.388	4471.410	0.997	0.000	0.000 – 0.000
	100≤x < 500	−15.838	4471.410	0.997	0.000	0.000 – 0.000
	500≤x < 1000	−18.948	4471.409	0.997	0.000	0.000 – 0.000
#Sample Type	Ectoparasite			0.000		
	Oral	−0.208	0.631	0.742	0.813	0.236 – 2.797
	Urine	0.504	0.798	0.528	1.655	0.346 – 7.913
	Guano	−2.435	0.575	0.000	0.088	0.028 – 0.270
##Roost type	Post-natal			0.159		
	Early-hibernation	−1.127	2.237	0.614	0.324	0.004 – 25.969
	End-hibernation	0.427	3.781	0.910	1.533	0.001 – 2534.608
	Diurnal roost	−3.094	1.349	0.022	0.045	0.003 – 0.638
	Pre-natal	−25.791	3161.408	0.993	0.000	0.000 – 0.000
###Species	*Rhinolophus ferrumequinum*			0.021		
	Miniopterus fuliginosus	0.519	0.776	0.503	1.681	0.368 – 7.685
	Murina hilgendorfi	−0.354	1.544	0.818	0.702	0.034 – 14.466
	Myotis aurascens	0.633	1.584	0.689	1.884	0.084 – 42.046
	Myotis bombinus	0.749	1.111	0.500	2.115	0.240 – 18.676
	Myotis macrodactylus	−1.721	0.882	0.051	0.179	0.032 – 1.007
	Myotis petax	−2.402	1.454	0.099	0.091	0.005 – 1.565
	Pipistrellus abramus	18.154	4349.697	0.997	76573581.338	0.000 – 0.000
####Sex	Male	0.530	0.280	0.058	1.699	0.982 – 2.942
#####BCI	BCI	2.108	3.783	0.577	8.233	0.005 – 13668.283

*Year: Year of bat sample collection; **Quarter: Quarter of bat sample collection; ***Location: Location of bat sample collection.
****Total No. of Bat: Total number of bats in the sample collection location; *****No. species: Number of bat species in the habitat at the time of sample collection; ******No. bats per one colony: Size of the bat colony at the time of sample collection.
#Sample type: Type of bat sample; ##Roost type: Habitat condition at the bat sample collection location; ###Species: Bat species of the sample; ####Sex: Gender of the bat; #####Body Condition Index (BCI): Body Condition Index of the Bat. Significant data are highlighted in yellow.

the "Ectoparasite" samples and "Guano" samples. In addition, the "species" variable "*Rhinolophus ferrumequinum*" was significantly correlated with coronavirus positivity (Table 2).

Discussion

Despite the lack of clarity relating to the origins of several viruses that posed a threat to humans, it has been established that most of these viruses are closely linked to spillover events occurring via interspecies transmission among wild animals or between animals and humans. As a result, research efforts are ongoing on viruses present in various wild animals and their zoonotic potential. Furthermore, it has now become imperative to conduct comprehensive analyses of the interactions between viruses and hosts at the interface between humans and animals [26]. Moreover, efforts are underway to examine the relationship between the ecology of bats and viruses, as research using various bat virus models have demonstrated that alterations in bat ecology are associated with the spillover of pathogens [12,27–29]. In case of bat coronaviruses, we need to investigate the coronavirus infection and transmission pattern among bat hosts. While recent studies have revealed that co-infection and spillover of viruses can occur among bats [30], additional ecological information on which host factors are correlated with the introduction and circulation of viruses among bat populations could facilitate the development of strategic measures, such as guiding targeted surveillance or identifying risk factors, to address the potential for zoonotic spillover.

In this study, only alphacoronaviruses were detected in 4.86% (75/1,543) of the samples, which were obtained

from bat habitats in 6 out of 7 regions in Korea using RDRP-based RT-nested PCR. In the previous reports, although there were both of alpha- and betacoronaviruses circulating in Korean bats [10,13,31–33], betacoronaviruses were not frequently detected as alphacoronaviruses. That might be able to substantiate consistent the findings in this study. Based on the detection rate of coronaviruses, the overall coronavirus-positive rates tended to surge in a specific period, indicating periodic fluctuation. Notably, when the coronavirus-positive samples were sequenced and phylogenetically analyzed, host-specific circulation pattern of bat coronaviruses became evident (Figure 2). Alphacoronaviruses from *R. ferrumequinum* were mostly detected in the B2 clade, while those from *Miniopterus fuliginosus* were detected in the M. ful/Japan and B15–41 clades. Alphacoronaviruses from *Myotis sp*. were mostly detected in clades, B1, and Myosis/China. Although Betacoronaviruses were not detected in this study, previous papers have reported that SARS-related coronaviruses were mostly detected in *R. ferrumequinum*, Korea [10,13]. Several evidences for bat species or genus-specific coronavirus circulation have been reported [34–36]. Although bats are known as reservoirs for recent novel coronaviruses in human, considering the 1,400 bat species on earth [37], it would be important to study which species of bats harbor specific types of coronaviruses and then identifying viruses with spillover potential among them. Thus, specific species of bats that are carriers of potential viruses should be further studied with regard to their distribution, migration patterns, and interactions with other species.

Under the natural conditions, it is not clearly demonstrated whether coronavirus-infected bats display clinical symptoms. The indicators of clinical symptoms are diverse, such as body temperature, body weight, coughs, and diarrhea. Among the clinical indicators, body weight was the easiest to measure in bats. However, factors such as bat species and age caused variations in body weight, making it insufficient to measure clinical changes based solely on weight. Therefore, due to the limited circumstances for studying wild animals, we adopted the Body Condition Index (BCI) to measure potential clinical changes caused by coronavirus infection in the study bats. Thus, in this study, we attempted to measure BCI of the captured bats and compared them between the coronavirus-positive and negative groups. The average BCI at different sampling times between the two groups were not significantly different. Although species-specific (*R. ferrumequinum*; P-value 0.004, *M. fuliginosus*; P-value 0.022, *M. macrodactylus*; P-value 0.028) differences of BCIs between two groups were observed, the apparent evidence for the clinical symptoms, such as BCI decrease was not found, as higher BCI in the coronavirus-positive group was observed in *R. ferrumequinum*. Although the BCI was affected by the age of bats and other factors such as their nutritional status, it may be considered that the coronavirus infection in the natural bats does not affect the BCIs. Referring to a previous report that experimental infection of bat coronaviruses in *L. rousette* bats did not show any clinical signs [7], clinical symptoms arising from infection of alphacoronaviruses circulating in Korean bats is not likely. However, additional factors such as body temperature should be studied to confirm the minor clinical signs, such as changes in BCI, caused by coronavirus infection in bats under natural conditions.

Notably, in this study, we detected the presence of coronaviruses in a subset of the 294 ectoparasites collected from captured bats, suggesting a potential bidirectional transmission mechanism between the bats and their ectoparasites. External parasites were first classified morphologically. Among them, the external parasites that tested positive for coronavirus were classified into two species morphologically. Similarly, genetic analysis of the external parasites that tested positive for coronavirus also classified them into two species, *Megastrebla* and *Spinturnix* genera. Remarkably, two coronavirus-positive ectoparasites were collected from the bats positive for coronavirus as well. Sequence comparison of partial RDRP sequences demonstrated genetic relatedness, indicating a potential role of ectoparasites in the transmission of bat coronaviruses. However, there are a few studies on coronaviruses in bat-associated ectoparasites, but their findings are not similar. There are several indirect evidence of the potential role of ectoparasites in the coronavirus transmission. The ectoparasite loads were correlated with coronavirus infection in Noack's roundleaf bats [38]. The potential binding of ACE in Arthropod ectoparasites to SARS-CoV-2 was inferred, but there was no infection [39]. Collectively, our finding may underscore the importance of investigating ectoparasites as vectors or fomites for coronaviruses transmission among bats, albeit additional studies are required on this issue.

Interestingly, during sampling, we could recapture several bats and test them for coronavirus again. In our findings, temporal dynamics were evident, with five bats showing absence of coronaviruses in certain subsequent months, followed by positive findings later. These observations underline the complexity of viral persistence and shedding within bat populations. Importantly, among the five cases of recapture bats, a *R. ferrumequinum* bat (E001270) exhibited coronavirus presence in both March and December 2021, with

a 72.34% sequence identity in the partial RDRP region, belonging to difference clades in the phylogenetic analysis. The concept of cross-protection may be considered when analyzing these patterns. While cross-protection can be effective against closely related viruses, genetic differences can limit its efficacy. As experienced in SARS-CoV-2 pandemic, rapid viral evolution and genetic differences can compromise cross-protection against new variants [40]. Similarly, a report suggested that immunity to a specific coronavirus may not extend to other coronaviruses due to genetic and antigenic differences [41]. Like in human, in bats, even when immunity develops against a certain coronavirus infection, new variants or new species of coronaviruses can still cause infections. These findings shed light on the intricate patterns of coronavirus recurrence, species-specific differences, and temporal trends, thereby providing insights into diverse coronavirus persistence among a bat population.

The collected bat ecological data were analyzed by binary logistic regression analysis after setting the positive status of coronavirus in bats as dependent variables, and the year, quarter, location, total number of bats, number of species in habitat, number of bats/colony, sample type, roost type, species, sex, and BCI as independent variables. The bat ecological factors that were significantly associated with coronavirus-positive cases were sample type, roost type, and species, but other factors were found to be non-significant. In case of roost-type variable, we categorized the roost type into early hibernation, end-hibernation, diurnal roost, and pre-natal and post-natal based on the bat ecology. The diurnal roost was demonstrated to be significantly associated with the coronavirus-positive samples. However, the odds ratio was 0.045, which meant that coronavirus-positive samples were not higher than for the post-natal cases. As bats tend to move around and change their habitats before breeding and hibernation, comingling of different bat colonies during this time may affect coronaviruses circulation [42], coronavirus-positive rate during diurnal roosting period seemed relatively low when compared to the breeding and hibernation periods. In this study, sample type was found to be a significant variable for coronavirus positivity. Especially, guano and ectoparasites were significantly associated with coronavirus positivity. In a previous meta-analysis, the detection of coronavirus was maximized in stool samples collected from workplace settings [43]. However, in this study, the odds ratio of guano was 0.088, which meant relatively lower coronavirus-positive rate. Previous studies on Nipahvirus and Hendravirus, which belong to the *Paramyxoviridae* family, have shown that virus levels are significantly higher in pregnant females [44,45]. Additionally, Hendravirus is horizontally transmitted through feces, urine, and saliva, and factors such as habitat changes, climate variations, and shifts in food availability are known to influence infection dynamics [44]. These findings may provide evidence to explain why our results differ from previous studies. Therefore, additional studies on coronavirus tropism and shedding from infected bats should be conducted, incorporating factors such as pregnancy status, habitat changes, and other environmental influences.

In conclusion, this study provided comprehensive information on the relationship between coronaviruses and bat ecology. Different bat species exhibited distinct coronavirus circulation patterns. The clinical symptoms in the coronavirus-positive bats were not clearly evident based on the BCI. Coronaviruses were detected in two bat ectoparasites, suggesting additional study on their potential role for the coronavirus transmission. The recaptured bats displayed dynamic coronavirus presence patterns such as reinfection with different coronavirus in the same individual. Ecological factors linked to coronavirus presence were analyzed, with the roost type, sample type, and bat species are significant factors. The study emphasizes comprehensive investigation into virus transmission dynamics within bat populations linked with bat ecology to understand their potential risk for future spillover events.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Bio & Medical Technology Development Program through National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) [2021M3A9I2080494 & 2021M3E5E3083402] and the National Institute of Ecology [NIE-B-2023-38]. This work is also supported by the KRIBB initiative program (KGM1262511) of Korea Research Institutes of Bioscience and Biotechnology, and funded by the Ministry of Education of Korea [2020R1A6A1A06046235].

Data availability statement

Data that support the findings of this study have been deposited in GenBank with the accession number OQ401100 to OQ401158 and OR700183 to OR700189. All data generated or analyzed during this study are deposited in figshare, DOI: https://doi.org/10.6084/m9.figshare.26548369

Author contributions

Conceptualization: H.K.K. and S.S.K. Methodology: M.C.K., S.S.J., H.A.L., H.Y.K., D.Y.M., T.V.L., and J.Y.N. Investigation: M.C.K., S.S.J., S.W.Y., D.G.J., S.S.K., H.K.K. Visualization: M.C.K., S.S.K., T.V.L., and H.K.K. Resources (sampling): S.S.K., K.K., T.W.L., and Y.G.C. Funding acquisition: H.K.K., S.S.K., and D.G.J. Project administration: H.K.K. and S.S.K. Supervision: H.K.K. and S.S.K. Writing, original draft: M.C.K., S.S.K., and H.K.K. Writing, review, and editing: J.Y.N., T.V.L., S.W.Y., and D.G.J. All authors have read and approved the final work.

Ethics declaration

This study was approved by the Research Planning Review Committee of the National Institute of Ecology (NIEIACUC-2021-001). We declare that we adhered to the Wildlife Protection and Management Act of Korea as well as the Institutional Research Ethics Regulations and Guidelines. All handling and sampling permissions were obtained from the seven corresponding local governments on each sampling year. We have adhered to ARRIVE guideline which checklist is also uploaded.

ORCID

Hye Kwon Kim http://orcid.org/0000-0003-3458-3403

References

[1] Letko M, Seifert SN, Olival KJ, et al. Bat-borne virus diversity, spillover and emergence. Nat Rev Microbiol. 2020;18(8):461–471. doi: 10.1038/s41579-020-0394-z

[2] Mbu'u CM, Mbacham WF, Gontao P, et al. Henipaviruses at the interface between bats, livestock and human population in Africa. In: Vector borne and zoonotic diseases. Vol. 19. Larchmont, N.Y.; 2019. p 455–465. doi: 10.1089/vbz.2018.2365

[3] Li W, Shi Z, Yu M, et al. Bats are natural reservoirs of SARS-like coronaviruses. In: Science. Vol. 310. New York, N.Y.; 2005. p 676–679. doi: 10.1126/science.1118391

[4] Memish ZA, Mishra N, Olival KJ, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis. 2013;19(11):1819–1823. doi: 10.3201/eid1911.131172

[5] Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270–273. doi: 10.1038/s41586-020-2012-7

[6] Ruiz-Aravena M, McKee C, Gamble A, et al. Author correction: ecology, evolution and spillover of coronaviruses from bats. Nat Rev Microbiol. 2022;20(5):315. doi: 10.1038/s41579-022-00691-3

[7] Watanabe S, Masangkay JS, Nagata N, et al. Bat coronaviruses and experimental infection of bats, the Philippines. Emerg Infect Dis. 2010;16(8):1217–1223. doi: 10.3201/eid1608.100208

[8] Bosco-Lauth AM, Porter SM, Fox KA, et al. Experimental infection of Brazilian free-tailed bats (tadarida brasiliensis) with two strains of SARS-CoV-2. Viruses. 2022;14(8):1809. doi: 10.3390/v14081809

[9] Hall JS, Knowles S, Nashold SW, et al. Experimental challenge of a North American bat species, big brown bat (Eptesicus fuscus), with SARS-CoV-2. Transbound Emerg Dis. 2021;68(6):3443–3452. doi: 10.1111/tbed.13949

[10] Lo VT, Yoon SW, Noh JY, et al. Long-term surveillance of bat coronaviruses in Korea: diversity and distribution pattern. Transbound Emerg Dis. 2020;67(6):2839–2848. doi: 10.1111/tbed.13653

[11] Warmuth VM, Metzler D, Zamora-Gutierrez V. Human disturbance increases coronavirus prevalence in bats. Sci Adv. 2023;9(13):eadd0688. doi: 10.1126/sciadv.add0688

[12] Eby P, Peel AJ, Hoegh A, et al. Pathogen spillover driven by rapid changes in bat ecology. Nature. 2023;613(7943):340–344. doi: 10.1038/s41586-022-05506-2

[13] Loh EH, Nava A, Murray KA, et al. Prevalence of bat viruses associated with land-use change in the Atlantic Forest, Brazil. Front Cell Infect Microbiol. 2022;12:921950. doi: 10.3389/fcimb.2022.921950

[14] Gonzalez V, Banerjee A. Molecular, ecological, and behavioral drivers of the bat-virus relationship. iScience. 2022;25(8):104779. doi: 10.1016/j.isci.2022.104779

[15] Becker DJ, Albery GF, Sjodin AR, et al. Optimising predictive models to prioritise viral discovery in zoonotic reservoirs. The Lancet Microbe. 2022;3(8):e625–e637. doi: 10.1016/S2666-5247(21)00245-7

[16] Becker DJ, Eby P, Madden W, et al. Ecological conditions predict the intensity of Hendra virus excretion over space and time from bat reservoir hosts. Ecol Lett. 2023;26(1):23–36. doi: 10.1111/ele.14007

[17] Cappelle J, Furey N, Hoem T, et al. Longitudinal monitoring in Cambodia suggests higher circulation of alpha and betacoronaviruses in juvenile and immature bats of three species. Sci Rep. 2021;11(1):24145. doi: 10.1038/s41598-021-03169-z

[18] Yoon MH, Han SH, Oh HS, et al. The mammals of Korea. dongbang media. Korean. 2004. Available from: http://www.koreaa2z.com/viewer.php?seq=11

[19] National Institute of Biological Resources. National institute of biological resources (NIBR) national list of species of Korea, vertebrates. 2012. Available from: https://www.nibr.go.kr/aiibook/ecatalog5.jsp?Dir=34&catimage=&callmode=admin

[20] Jo YS, Baccus JT, Koprowski JL. Mammals of Korea. Incheon, Republic of Korea: National Institute of Biological Resource; 2019.

[21]). Ecological and behavioral methods for the study of bats. In: Gannon WL, Kunz TH, and Parsons S, editors. Journal of mammalogy. 2nd ed. Vol. 92. Baltimore (MD): Johns Hopkins University Press; 2009 [2011 Apr 15, Pages 475–478, p 901 pp. ISBN doi: 10.1644/jmammal/92-2-475 978-0-8018–9147–2, price (hard-bound), $100.00, 2.

[22] Abouelhassan EM, El-Gawady HM, Abdel-Aal AA, et al. Comparison of some molecular markers for tick species identification. J Arthropod Borne Dis. 2019;13(2):153–164. doi: 10.18502/jad.v13i2.1241

[23] Hall TA. BioEdit: a user-friendly biological sequence alignment Editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–98. Available from: https://api.semanticscholar.org/CorpusID:82421255
[24] Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22(22):4673–4680. doi: 10.1093/nar/22.22.4673
[25] Page RD. Visualizing phylogenetic trees using TreeView. Current protocols in bioinformatics, Chapter 6 Curr Protoc Bioinformatics. 2002;1(1). doi: 10.1002/0471250953.bi0602s01
[26] Guth S, Mollentze N, Renault K, et al. Bats host the most virulent—but not the most dangerous—zoonotic viruses. Proc Natl Acad Sci USA. 2022;119(14): e2113628119. doi: 10.1073/pnas.2113628119
[27] Joffrin L, Hoarau AOG, Lagadec E, et al. Seasonality of coronavirus shedding in tropical bats. R Soc Open Sci. 2022;9(2):211600. doi: 10.1098/rsos.211600
[28] Lunn TJ, Peel AJ, McCallum H, et al. Spatial dynamics of pathogen transmission in communally roosting species: impacts of changing habitats on bat-virus dynamics. J Anim Ecol. 2021;90(11):2609–2622. doi: 10.1111/1365-2656.13566
[29] Meza DK, Mollentze N, Broos A, et al. Ecological determinants of rabies virus dynamics in vampire bats and spillover to livestock. Proc Biol Sci. 2022;289 (1982):20220860. doi: 10.1098/rspb.2022.0860
[30] Wang J, Pan YF, Yang LF, et al. Individual bat virome analysis reveals co-infection and spillover among bats and virus zoonotic potential. Nat Commun. 2023;14 (1):4079. doi: 10.1038/s41467-023-39835-1
[31] Kim HK, Yoon SW, Kim DJ, et al. Detection of severe acute respiratory syndrome-like, middle east respiratory syndrome-like bat coronaviruses and group H rotavirus in faeces of Korean bats. Transbound Emerg Dis. 2016;63(4):365–372. doi: 10.1111/tbed.12515
[32] Lee S, Jo SD, Son K, et al. Genetic characteristics of coronaviruses from Korean bats in 2016. Microb Ecol. 2018;75(1):174–182. doi: 10.1007/s00248-017-1033-8
[33] Kim Y, Son K, Kim YS, et al. Complete genome analysis of a SARS-like bat coronavirus identified in the Republic of Korea. Virus Genes. 2019;55(4):545–549. doi: 10.1007/s11262-019-01668-w
[34] Lazov CM, Chriél M, Baagøe HJ, et al. Detection and characterization of distinct alphacoronaviruses in five different bat species in Denmark. Viruses. 2018;10 (9):486. doi: 10.3390/v10090486
[35] Caraballo DA, Sabio MS, Colombo VC, et al. The role of molossidae and vespertilionidae in shaping the diversity of alphacoronaviruses in the Americas. Microbiol Spectr. 2022;10(6):e0314322. doi: 10.1128/spectrum.03143-22
[36] Apaa T, Withers AJ, Staley C, et al. Sarbecoviruses of British horseshoe bats; sequence variation and epidemiology. J Gen Virol. 2023;104(6):10 .1099/jgv.0.001859. doi: 10.1099/jgv.0.001859
[37] Don EW, Russell AM. Handbook of the mammals of the world, volume 9: bats. Barcelona, ES: Lynx Edictions; 2019.
[38] Baldwin HJ. Epidemiology and ecology of virus and host: bats and coronaviruses in Ghana, West Africa [thesis]. Macquarie University; 2016. doi: 10.25949/19432703.v1
[39] Lam SD, Ashford P, Díaz-Sánchez S, et al. Arthropod ectoparasites have potential to bind SARS-CoV-2 via ACE. Viruses. 2021;13(4):708. doi: 10.3390/v13040708
[40] Mlcochova P, Kemp SA, Dhar MS, et al. SARS-CoV-2 B.1.617.2 delta variant replication and immune evasion. Nature. 2021;599(7883):114–119. doi: 10.1038/s41586-021-03944-y
[41] Callow KA, Parry HF, Sergeant M, et al. The time course of the immune response to experimental coronavirus infection of man. Epidemiol Infect. 1990;105 (2):435–446. doi: 10.1017/s0950268800048019
[42] Kerth G. Causes and consequences of sociality in bats. BioScience. 2008;58(8):737–746. doi: 10.1641/B580810
[43] Cohen LE, Fagre AC, Chen B, et al. Coronavirus sampling and surveillance in bats from 1996–2019: a systematic review and meta-analysis. Nat Microbiol. 2023;8(6):1176–1186. doi: 10.1038/s41564-023-01375-1
[44] Boardman WSJ, Baker ML, Boyd V, et al. Seroprevalence of three paramyxoviruses; Hendra virus, Tioman virus, cedar virus and a rhabdovirus, Australian bat lyssavirus, in a range expanding fruit bat, the Grey-headed flying fox (pteropus poliocephalus). PLOS ONE. 2020;15(5):e0232339. doi: 10.1371/journal.pone.0232339
[45] Plowright RK, Field HE, Smith C, et al. Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (pteropus scapulatus). Proc Biol Sci. 2008;275(1636):861–869. doi: 10.1098/rspb.2007.1260