Wildlife in Cameroon harbor diverse coronaviruses, including many closely related to human coronavirus 229E

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Abstract

Zoonotic spillover of animal viruses into human populations is a continuous and increasing public health risk. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) highlights the global impact of emergence. Considering the history and diversity of coronaviruses (CoVs), especially in bats, SARS-CoV-2 will likely not be the last to spillover from animals into human populations. We sampled and tested wildlife in the Central African country Cameroon to determine which CoVs are circulating and how they relate to previously detected human and animal CoVs. We collected animal and ecological data at sampling locations and used family-level consensus PCR combined with amplicon sequencing for virus detection. Between 2003 and 2018, samples were collected from 6,580 animals of several different orders. CoV RNA was detected in 175 bats, a civet, and a shrew. The CoV RNAs detected in the bats represented 17 different genetic clusters, coinciding with alpha ($n = 8$) and beta ($n = 9$) CoVs. Sequences resembling human CoV-229E (HCoV-229E) were found in 40 *Hipposideridae* bats. Phylogenetic analyses place the human-derived HCoV-229E isolates closest to those from camels in terms of the S and N genes but closest to isolates from bats for the envelope, membrane, and RNA-dependent RNA polymerase genes. The CoV RNA positivity rate in bats varied significantly (P < 0.001) between the wet (8.2 per cent) and dry seasons (4.5 per cent). Most sampled species accordingly had a wet season high and dry season low, while for some the opposite was found. Eight of the suspected CoV species of which we detected RNA appear to be entirely novel CoV species, which suggests that CoV diversity in African wildlife is still rather poorly understood. The detection of multiple different variants of HCoV-229E-like viruses supports the bat reservoir hypothesis for this virus, with the phylogenetic results casting some doubt on camels as an intermediate host. The findings also support the previously proposed influence of ecological factors on CoV circulation, indicating a high level of underlying complexity to the viral ecology. These results indicate the importance of investing in surveillance activities among wild animals to detect all potential threats as well as sentinel surveillance among exposed humans to determine emerging threats.

Key words: Coronavirus; cameroon; bats; HCoV-229E; seasonality; wildlife

1. Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has highlighted the inherent risks and potential consequences of pathogen spillovers from animal reservoirs into the human populations. Animals are known to be the reservoir for many human diseases historically and contemporarily as exemplified by zoonotic diseases, such as rabies, brucellosis, bubonic plague, and trichinellosis (Shope 1982; Weiss et al. 2003; Wolfe et al. 2007; Gottstein et al. 2009; Drancourt and Raoult 2016; Cross et al. 2019). Humans are often considered an accidental host for zoonotic diseases in this context, even if human-to-human transmission is possible. This understanding has shifted

with the advent, and increased availability, of genetic characterization of pathogens, and humans are now often considered opportunistic rather than accidental hosts. This is an especially apt description for viruses, since we now know that a significant number of pathogens commonly referred to as 'human viruses' did not originally evolve with humans but spilled over from animals more recently and subsequently adapted to humans (Weiss et al. 2003; Wolfe et al. 2007). The best example for this may be HIV, which originated from multiple non-human primate (NHP) spillover events in Africa during the early 20th century, and it also applies to measles virus, influenza A viruses, and others (Weiss et al. 2003; Sharp and Hahn 2011). While these and SARS-CoV-2 are some of the most publicized examples, there is a general trend of increasing infectious diseases outbreaks in humans, in particular, viral and zoonotic agents, over the past decades, potentially due to factors such as land use and climate change, population growth, and increased international trade and mobility (Jones et al. 2008; Smith et al. 2014; Allen et al. 2017).

With the global SARS-CoV-2 pandemic, we are witnessing such a post-spillover adaptation in real time, and there is strong evidence that this is not the first time an animal coronavirus (CoV) has gone through this process. While primary attention has previously focused on SARS-CoV, Middle East Respiratory Syndrome (MERS) CoV, and closely related CoVs, it has become clear that the four CoVs that are associated with the common cold (HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1), all likely derived from animal CoVs in the previous decades to centuries (Drosten et al. 2003; Kan et al. 2005; Yip et al. 2009; Drexler et al. 2010; Zaki et al. 2012; Huynh et al. 2012; Corman et al. 2014, 2015, 2018; Forni et al. 2017; Cui et al. 2019). Unlike SARS-CoV-1 and MERS-CoV, these four established themselves in the human population in a process that may have been similar to the current SARS-CoV-2 pandemic, albeit likely much slower. Genetic analysis suggests that the two alpha CoVs HCoV-NL63 and HCoV-229E originated in bats, like SARS-CoVs and MERS-CoV, while the beta CoVs HCoV-OC43 and HCoV-HKU1 likely originated in rodents; in either case with or without a potential intermediate host (Pfefferle et al. 2009; Tao et al. 2017; Zhou et al. 2020). The fact that we know of three CoVs that spilled over into humans in just the past two decades and that spillovers of the other four can be traced back to recent centuries suggests that these events are common, especially since it is estimated that only a minority of spillover events lead to continued transmission and detection in humans (Glennon et al. 2019; Letko et al. 2020). CoV spillovers will thus likely continue to occur in the future. This indicates that a better understanding of animal CoVs will be useful to determine spillover risks and the biological mechanisms and drivers for diversification and spillover and to develop appropriate prevention, mitigation, and treatment strategies for future CoV spillovers.

It is generally accepted that there is a direct link between a close genetic relationship of host species and the likelihood of interspecies transmission; however, considering the biology of influenza A viruses, for example, where we see spillover from pigs and birds into humans, or CoVs where bats, rodents, camels, cows, and civets may play a role, it is by no means a hard rule (Kan et al. 2005; Parrish et al. 2008; Dennehy 2017). With less closely related host species, the risks are more difficult to determine, but much emphasis has recently been placed on host diversity as an indicator for viral diversity, and the role of contact rates (Wolfe et al. 2005; Pike et al. 2010; Maganga et al. 2014; Anthony et al. 2017; Dennehy 2017; Leopardi et al. 2018; Ntumvi et al. 2021). Host diversity likely results in more viruses circulating in a biome and also provides more opportunities for interspecies transmission, host plasticity, and viral recombination (Dennehy 2017). Host

plasticity in animals may in turn be a predictor for a virus' ability to be transmitted from human to human and hence a major risk factor (Kreuder Johnson et al. 2015). Consequently, regions with a high biodiversity, such as large parts of Central Africa, South America, and Southeast Asia, may be considered hot spots for spillover (Jones et al. 2008; Allen et al. 2017). Reports from several African countries suggest that there are many CoVs circulating, primarily in bats, including species related to pathogens such as SARS-CoV-1, MERS-CoV, HCoV-229E, and HCoV-NL63 (Tong et al. 2009; Tao et al. 2012, 2017; Annan et al. 2013; Geldenhuys et al. 2013, 2018, 2021; Razanajatovo et al. 2015; Anthony et al. 2017; Markotter et al. 2019; Nziza et al. 2019; Maganga et al. 2020; Kumakamba et al. 2021).

While the viral genome provides a lot of information about viruses, their origins, and their hosts, other factors also need to be considered when evaluating risks. Direct or indirect human–animal interaction is a prerequisite for zoonotic transmission, but human and animal behaviors and ecologies can play key roles in this process that may involve multiple steps of interspecies transmission and adaptation (Wolfe et al. 2005; Wolfe et al. 2007; Pike et al. 2010; Maganga et al. 2014; Euren et al. 2020; Kumakamba et al. 2021; Ntumvi et al. 2021). Previous studies have, for example, identified a high host plasticity for certain bat CoVs, indicating that these may potentially pose a higher zoonotic risk than those primarily adapted to a single host (Anthony et al. 2017). Other factors influencing transmission may include the type of human–animal interface and seasonal fluctuations of CoV circulation as observed in bat populations (Montecino-Latorre et al. 2020; Kumakamba et al. 2021; Grange et al. 2021). Although important, data on many of these factors are still limited and needs further exploration. Studying CoV diversity in Africa promises rich data that could improve our understanding of their biology, evolutionary history, and risks for humans.

The Central African country Cameroon, which includes some of the northern part of the Congo Basin, where HIV is believed to have spilled over into humans, is rich in biodiversity, with wildlife interaction being common for a large part of the rural population. Increased bushmeat trade and diverse wildlife living in close proximity with human populations makes some of these areas hotspots for high-risk interfaces between animals and humans (Wolfe et al. 2005; Mickleburgh et al. 2009; Saylors 2021).

Our goal was to determine what CoVs are circulating in wild animals, including rodents, bats, and NHPs, and assess if key ecological factors may influence the rate of CoV detection and thus the exposure risk.

2. Materials and methods

2.1 Sample and field data collection

Sample acquisition methods differed depending on the species and interface. Animals in peridomestic settings were captured and released after sampling (bats, rodents, and shrews only), while samples from the (bushmeat) value chain were collected from freshly killed animals voluntarily provided by local hunters upon their return to the village following hunting or by vendors at markets. Non-invasive fecal samples were collected from free-ranging NHPs, while some NHP samples, such as blood or serum, were collected during routine veterinary exams in zoos and wildlife sanctuaries. To avoid incentivizing hunting, hunters and vendors were not compensated. Identification was done in the field by trained field ecologists, as well as retrospectively based on field guides and other resources, including those by Kingdon and Monadjem (Kingdon 2005; Monadjem et al. 2010, 2015). Oral and rectal swab samples were collected into individual

2.0-ml screw-top cryotubes containing 1.5 ml of either Universal Viral Transport Medium (BD), RNA later, lysis buffer, or Trizol® (Invitrogen), while pea-sized tissue samples were placed in 1.5-ml screw-top cryotubes containing 500 μl of either RNA later or lysis buffer (Qiagen), or without medium. Specimen collection was approved by an Institutional Animal Care and Use Committee of University of California (UC) Davis (#16067, # 17803, and # 19300), Johns Hopkins University (JHU) (ACR #2007-110-03, #2007-110-02 and #2007-110-11), and University of California Los Angeles (UCLA) (Protocols # FS03M221 and FS06H205) and the Government of Cameroon. All samples were stored in liquid nitrogen as soon as practical, before being stored long term in freezers at −80°C. Sample collection staff were trained in safe collection techniques in collaboration with representatives from Ministry of Fisheries Livestock Animal Industries (MINEPIA), the Ministry of Forestry and Wildlife (MINFOF), and the Ministry of Environment Nature Protection and Sustainable Development (MINEPDED) and wore dedicated clothing, N95 masks, nitrile gloves, and protective eyewear during animal capture, handling, and sampling.

2.2 Sample processing

All laboratory work was carried out at Cameroon's Military Health Research Center (CRESAR). RNA was extracted either manually using Trizol®, with an Qiagen AllPrep kit (tissue), Qiagen Viral RNA Mini Kit (swabs collected prior to 2014), or with a Zymo Direct-zol RNA kit (swabs collected after 2014) and stored at −80°C. Afterwards, RNA was converted into cDNA using a GoScript™ Reverse Transcription kit (Promega) and stored at −20°C until analysis. Two conventional nested broad range PCR assays, designed based on different sequence sets, both targeting different conserved regions within the RNA-dependent RNA polymerase gene (RdRp) were used successfully to screen samples of bats, rodents, and other animals (and humans) for CoV RNA (Anthony et al. 2017; McIver et al. 2020; Kumakamba et al. 2021; Li et al. 2021). The first PCR amplifies a product of approximately 286 nucleotides between the primer binding sites and was specifically designed for the detection of a broad range of CoVs (Quan et al. 2010). The second PCR was used in two modified versions, with one of them specifically targeting a broad range of CoVs in bats and the second one broadly targeting CoVs of other hosts (Watanabe et al. 2010). Both versions amplify 387 nucleotides between the primer binding sites. Follow-up PCRs were designed to amplify the 3′-end of the Spike (S), as well as the Envelope (E), Membrane (M), and Nucleoprotein (N) genes of CoVs related to HCoV-229E (Table 1).

PCR products were subjected to gel electrophoresis on a 1.5 per cent agarose gel to identify amplicons of the expected size. PCR was repeated for all samples where this was the case, and PCR products were excised. Amplified DNA was extracted using either the QIAquick Gel Extraction Kit (Qiagen) or the Wizard SV Gel and PCR Cleanup System (Promega) and sent for commercial Sanger sequencing at either GATC, First Base, or Macrogen. Extracts with low DNA concentrations were cloned prior to sequencing. All results from sequencing were analyzed in the Geneious 7.1 software, and primer trimmed consensus sequences compared to the GenBank database (BLAST N, NCBI). Samples where the PCR results could not be repeated, or where the sequencing did not yield interpretable sequences consistent with a CoV were counted as CoV RNA negative. All viral sequences obtained were deposited in the GenBank (Table 2, Supplement 1).

2.3 Phylogenetic analysis

To facilitate phylogenetic analysis, select sequences of published complete CoV genomes isolated from humans, bats, and other hosts, as well as partial sequences from CoVs closely related to those detected in this study were included. Novel Cameroonian sequences were included if they differed from others by at least 5 per cent. Multiple sequence alignments were made in Geneious (version 11.1.3, ClustalW Alignment). Bayesian phylogeny of the polymerase gene fragment was inferred using MrBayes (version 3.2) with the following parameters: Datatype = DNA, Nucmodel = 4by4, Nst = 1, Coavion = No, # States = 4, Rates = Equal, 2 runs, 4 chains of 10,000,000 generations. The sequence of an avian Gamma Coronavirus (NC_001451) served as outgroup to root the trees based on the RdRp PCR amplicons, while HCoV-NL63 (AY467487) served as outgroup for sequences related to HCoV-229E. Trees were sampled after every 1,000 steps during the process to monitor phylogenetic convergence, and the final average standard deviation of split frequencies was below the MrBayes-recommended final average <0.01 for all analyses (Ronquist et al. 2012). The first 10 per cent of the trees were discarded and the remaining ones combined using TreeAnnotator (version 2.5.1; http://beast.bio.ed.ac.uk) and displayed with FIGTREE (1.4.4; http://tree.bio.ed.ac.uk/) (Bouckaert et al. 2019).

2.4 Statistical analysis

Ecological data collected along with the samples obtained from bats were statistically analyzed in relation to the outcome of PCR tests. The variables included species, family, and suborder, age, and sex, and factors such as the interface of exposure with humans, and season (wet/dry) of sampling. The taxonomy information for the analysis was based in the Integrated Taxonomic Information System website (https://www.itis.gov/), age coded as either adult or non-adult, interface categorized as 'value chain' for animal samples obtained at markets or directly from hunters, 'tourism' for samples obtained at zoos and sanctuaries, and 'other peridomestic'. The seasons were defined as switching from wet to dry after November 15th and from dry to wet after March 15th of each year. All statistical data analyses were conducted using the statistical software Statistical Package for the Social Sciences version 26. At the univariate level, frequencies and percentages of the selected variables of interest (i.e. PCR test results, species, family, suborder, age, sex, interface, season, etc.) were generated. At the bivariate level, simple cross-tabulation, chi-square tests have been used to determine the statistical association (which are considered statistically significant at 5 per cent level) between outcome and predictor variables. Here, the PCR test result has been considered as the dependent variable, and species, family, suborder, age, sex, interface, and season have been considered as the predictor/independent variables.

3. Results

3.1 Sample set

A total of 11,474 samples from 6,580 animals of several different orders were collected between 2003 and 2018, covering all 10 regions of Cameroon (Fig. 1, Supplement 2). Animals sampled were largely rodents (2,740/41.6 per cent), bats (2,581/39.2 per cent), and primates (1,006/15.3 per cent) representing 28 rodent species, 50 bat species, and 24 primate species. Samples were also collected from 159 Eulipotyphla (3 species), 38 pangolins (1 species), 37 carnivores (3 species), 17 even-toed ungulates (4 species), and 2 hyraxes (1 species) (Supplement 3). Samples were predominantly oral (5,214) and rectal (4,818) swabs but also included tissues, such as spleen (893), liver (93), lung (5), colon (4), small intestine (4), muscle (1), skin (1), and thyroid (1). Other sam-

Table 1. PCR primers and protocols.

PCR type and Target	Primers	Conditions
Nested family-level CoV PCR targeting a ∼300-nt region of the RdRp gene (Quan et al. 2010)	First round: CoV-FWD1: CGT TGG IAC WAA YBT VCC WYT ICA RBT RGG CoV-RVS1: GGT CAT KAT AGC RTC AVM ASW WGC NAC ATG Second round: CoV-FWD2: GGC WCC WCC HGG NGA RCA ATT CoV-RVS2: GGW AWC CCC AYT GYT GWA YRT C	First round: Initial denaturation at 95°C for 5 minutes, then 15 cycles of: 95°C for 30 seconds, 65°C for 30 seconds and 72°C for 45 seconds. This is followed by 40 cycles of: 95°C for 30 seconds, 50°C for 30 seconds and 72°C for 45 seconds. Final elongation at 72°C for 7 minutes. Second round: Initial denaturation at 95°C for 5 minutes, then 15 cycles of: 95°C for 30 seconds, 65°C for 30 seconds and 72°C for 45 seconds. This is followed by 35 cycles of: 95°C for 30 seconds, 50°C for 30 seconds and 72°C for 45 seconds. Final elongation at 72°C for 7 minutes.
Hemi-nested family-level CoV PCR targeting a ∼400-nt region of the RdRp gene (Watanabe et al. 2010)	First round: CoV-FWD3: GGT TGG GAY TAY CCH AAR TGT GA CoV-RVS3: CCA TCA TCA SWY RAA TCA TCA TA Second round: FWD4/Other: GAY TAY CCH AAR TGT GAU MGW GC CoV-RVS3: CCA TCA TCA SWY RAA TCA TCA TA	First round: Initial denaturation at 94°C for 2 minutes, then 35 cycles of: 94°C for 20 seconds, 50°C for 30 seconds and 72°C for 30 seconds. Final elongation at 72°C for 7 minutes. Second round: Initial denaturation at 94°C for 2 minutes, then 35 cycles of: 94°C for 20 seconds, 50°C for 30 seconds and 72°C for 30 seconds. Final elongation at 72°C for 7 minutes.
Hemi-nested family-level bat CoV PCR targeting a ∼400-nt region of the RdRp gene (Watanabe et al. 2010)	First round: CoV-FWD3: GGT TGG GAY TAY CCH AAR TGT GA CoV-RVS3: CCA TCA TCA SWY RAA TCA TCA TA Second round: CoV-FWD4/Bat: GAY TAY CCH AAR TGT GAY AGA GC CoV-RVS3: CCA TCA TCA SWY RAA TCA TCA TA	First round: Initial denaturation at 94°C for 2 minutes, then 35 cycles of: 94°C for 20 seconds, 50°C for 30 seconds and 72°C for 30 seconds. Final elongation at 72°C for 7 minutes. Second round: Initial denaturation at 94°C for 2 minutes, then 35 cycles of: 94°C for 20 seconds, 50°C for 30 seconds and 72°C for 30 seconds. Final elongation at 72°C for 7 minutes.
Species-level HCoV-229E PCR targeting a ∼900-nt region at the 3′-prime end of the S gene	29E-CoV-S-3prime fwd: GGT AGA TAG RCT KAT TAM TGG 229E-CoV-S-3prime rev: TCA ACG TCG TAA TAA GGA AG	Initial denaturation at 95°C for 3 minutes, then 40 cycles of: 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 1.5 minutes. Final elongation at 72°C for 5 minutes.
Species-level HCoV-229E PCR targeting a ∼700-nt region central in the S gene	229E-CoV-S-mid fwd: GTD GGT GCT ATG WTG TCT G 229E-CoV-S-mid rev: TCA GCA TCA GCR ACR CCH G	Initial denaturation at 95°C for 3 minutes, then 40 cycles of: 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 1.5 minutes. Final elongation at 72°C for 5 minutes.
Species-level HCoV-229E PCR targeting a ∼700-nt region central in the S gene	229E-CoV-S-cent fwd: TCA CTC CTT GYA ACC CAC CAG 229E-CoV-S-mid rev: TCA GCA TCA GCR ACR CCH G	Initial denaturation at 95°C for 3 minutes, then 40 cycles of: 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 1.5 minutes. Final elongation at 72°C for 5 minutes.
Species-level HCoV-229E PCR targeting a ∼700-nt region between the center and the 3′-prime end of the S gene	229E-CoV-S-link fwd: CTG GWC TTG GCA CTG TKG A 229E-CoV-S-link rev: CCR TCA GGA GCA GCA TTV AC	Initial denaturation at 95°C for 3 minutes, then 40 cycles of: 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 1.5 minutes. Final elongation at 72°C for 5 minutes.
Species-level HCoV-229E PCR targeting a ∼350-nt region of the M gene	229E-CoV-M fwd: GGC CAC TTG TAC TTG CWY 229E-CoV-M rev: TAG TAG TGC TCG GCA CGG CAA C	Initial denaturation at 95°C for 3 minutes, then 40 cycles of: 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 45 seconds. Final elongation at 72°C for 5 minutes.
Species-level HCoV-229E PCR targeting a ∼850-nt region of the N gene	229E-CoV-Nuc fwd: CCT TGG AAG GTG ATA CCT C 229E-CoV-Nuc rev: CAA ACA GCA TAG CAG CTG T	Initial denaturation at 95°C for 3 minutes, then 40 cycles of: 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 1.5 minutes. Final elongation at 72°C for 5 minutes.
Species-level HCoV-229E PCR targeting a ∼500-nt region of the M and N genes	229E-CoV-M/N-link fwd: TCC AAC AGG CAT CAC GGT GAC 229E-CoV-M/N-link rev: TCC TTA AAA GGG CCT GTT CC	Initial denaturation at 95°C for 3 minutes, then 40 cycles of: 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 1 minutes. Final elongation at 72°C for 5 minutes.
Species-level HCoV-229E PCR targeting a ∼1200-nt region around the E gene	229E-CoV-E+ fwd: GTC TTG CAT CTT CTA CTA GAG G 229E-CoV-E+ rev: GTA CCC CAA TTA GCC CAG G	Initial denaturation at 95°C for 3 minutes, then 40 cycles of: 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 2 minutes. Final elongation at 72°C for 5 minutes.

ple types included plasma (377), serum (20), and whole blood (1), as well as feces (36), genital swabs (5), and nasal swabs (1).

CoV RNA was detected in at least one sample with at least one PCR assay in 175 individual bats, 1 civet, and 1 shrew (Table 2).

Rectal swabs were CoV RNA positive in 129 instances, oral swabs in 71 instances, liver and spleen in 3 instances each, and plasma in 2 instances. The Watanabe PCR protocol produced 173 positive results, while the Quan PCR protocols produced 64 positive results.

Table 2. List of samples containing CoV RNA.

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
Bat CoV cluster 1 A (W)/Alpha CoV	CMAB71480/MT081987/ BtCoV/H.ruber/CMR/ CMAB71480r/2016	*Hipposideros ruber*/rectal swab	26 May 2016/South	99% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB71481/MT082023/ BtCoV/H.ruber/CMR/ CMAB71481r/2016	*Hipposideros ruber*/rectal swab	26 May 2016/South	99% Bat coronavirus isolate CS105 (MG963190)
	CMAB71535/MT221710/ BtCoV/Hip.sp/CMR/ CMAB71535r/2016	*Hipposideros* sp./rectal swab	26 May 2016/South	99% Bat coronavirus isolate CS105 (MG963190)
	CMAB71678/MT082019/ BtCoV/H.ruber/CMR/ CMAB71678r/2016	*Hipposideros ruber*/rectal swab	4 July 2016/South	98% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB71786/MT082020/ BtCoV/H.ruber/CMR/ CMAB71786r/2016	*Hipposideros ruber*/rectal swab	30 July 2016/South	99% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB71818/MT081983/ BtCoV/H.ruber/CMR/ CMAB71818r/2016	*Hipposideros ruber*/rectal swab	30 July 2016/South	99% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB72009/MT081994/ BtCoV/H.ruber/CMR/ CMAB72009r/2016	*Hipposideros ruber*/rectal swab	13 September 2016/South	99% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB72047/MT081996/ BtCoV/H.ruber/CMR/ CMAB72047r/2016	*Hipposideros ruber*/rectal swab	13 September 2016/South	99% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB72057/MT082030/ BtCoV/H.ruber/CMR/ CMAB72057r/2016	*Hipposideros ruber*/oral swab	13 September 2016/South	99% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB72143/MT082016/ BtCoV/H.ruber/CMR/ CMAB72143r/2016	*Hipposideros ruber*/rectal swab	29 October 2016/South	98% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB72642/MT082018/ BtCoV/H.ruber/CMR/ CMAB72642r/2017	*Hipposideros ruber*/rectal swab	18 February 2017/South	98% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB72694/MT082026/ BtCoV/H.ruber/CMR/ CMAB72694r/2017	*Hipposideros ruber*/rectal swab	21 February 2017/South	98% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB73354/MT082057/ BtCoV/H.ruber/CMR/ CMAB73354o/2017	*Hipposideros ruber*/oral swab	11 April 2017/South	98% Bat coronavirus isolate CS105 (MG963190)
	CMAB73357/MT082056/ BtCoV/H.ruber/CMR/ CMAB73357r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	98% Bat coronavirus isolate CS105 (MG963190)
	CMAB73364/MT082006/ BtCoV/H.ruber/CMR/ CMAB73364r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	98% Bat coronavirus isolate CS105 (MG963190)
	CMAB73367/MT082029/ BtCoV/H.ruber/CMR/ CMAB73367o/2017	*Hipposideros ruber*/oral swab	11 April 2017/South	98% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB73368/MT082008/ BtCoV/H.ruber/CMR/ CMAB73368r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	98% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB73376/MT082009/ BtCoV/H.ruber/CMR/ CMAB73376r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	98% Bat coronavirus isolate CS105 (MG963190)
	CMAB74746/MT082084/ BtCoV/H.ruber/CMR/ CMAB74746r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	99% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB74760/MT082078/ BtCoV/H.ruber/CMR/ CMAB74760r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	99% Bat coronavirus isolate 09GB0379 (MG963197)
	CMAB75005/MT082090/ BtCoV/H.curtus/CMR/ CMAB75005r/2018	*Hipposideros curtus*/rectal swab	30 May 2018/South	96% Bat coronavirus isolate CS105 (MG963190)

(continued)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	CMAB75013/MT082091/ BtCoV/H.ruber/CMR/ CMAB75013o/2018	*Hipposideros ruber*/oral swab	30 May 2018/South	98% Bat coronavirus isolate 10GB0309 (MG963199)
	CMAB75013/MT082092/ BtCoV/H.ruber/CMR/ CMAB75013r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	98% Bat coronavirus isolate 10GB0309 (MG963199)
	GVF-CM-ECO06296/ KX284955/BtCoV/H.caffer/ CMR/ECO06296r/2012	*Hipposideros caffer*/rectal swab	9 September 2012/Center	98% Bat coronavirus isolate 09GB0379 (MG963197)
Bat CoV cluster 1 B (W)/Alpha CoV	CMAB71642/MT081988/ BtCoV/H.ruber/CMR/ CMAB71642r/2016	*Hipposideros ruber*/rectal swab	4 July 2016/South	96% Bat coronavirus isolate 10GB0309 (MG963199)
	CMAB71651/MT081991/ BtCoV/H.ruber/CMR/ CMAB71651r/2016	*Hipposideros ruber*/rectal swab	4 July 2016/South	96% Bat coronavirus isolate 10GB0309 (MG963199)
	CMAB71675/MT082058/ BtCoV/H.ruber/CMR/ CMAB71675r/2016	*Hipposideros ruber*/rectal swab	4 July 2016/South	96% Bat coronavirus isolate 10GB0309 (MG963199)
	CMAB71800/MT082021/ BtCoV/H.ruber/CMR/ CMAB71800r/2016	*Hipposideros ruber*/rectal swab	30 July 2016/South	97% 229E-related bat coronavirus strain BtKY229E-8 (KY073748)
	CMAB72010/MT082012/ BtCoV/H.ruber/CMR/ CMAB72010r/2016	*Hipposideros ruber*/rectal swab	13 September 2016/South	98% Human coronavirus 229E PREDICT-ZB12046 (KX284928)
	CMAB72015/MT081993/ BtCoV/H.ruber/CMR/ CMAB72015r/2016	*Hipposideros ruber*/rectal swab	13 September 2016/South	99% Human coronavirus 229E PREDICT-ZB12046 (KX284928)
	CMAB72622/MT082053/ BtCoV/H.ruber/CMR/ CMAB72622r/2017	*Hipposideros ruber*/rectal swab	18 February 2017/South	98% Human coronavirus 229E PREDICT-ZB12046 (KX284928)
	CMAB73355/MT082055/ BtCoV/H.ruber/CMR/ CMAB73355r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	99% Human coronavirus 229E PREDICT-ZB12046 (KX284928)
	CMAB73356/MT082031/ BtCoV/H.ruber/CMR/ CMAB73356r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	99% Human coronavirus 229E PREDICT-ZB12046 (KX284928)
	CMAB73372/MT082010/ BtCoV/H.ruber/CMR/ CMAB73372r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	99% Human coronavirus 229E PREDICT-ZB12046 (KX284928)
	CMAB75014/MT082093/ BtCoV/H.ruber/CMR/ CMAB75014r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	99% Human coronavirus 229E PREDICT-ZB12046 (KX284928)
	CMAB75019/MT082081/ BtCoV/H.ruber/CMR/ CMAB75019r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	98% Human coronavirus 229E PREDICT-ZB12046 (KX284928)
Bat CoV cluster 1 C (Q)/Alpha CoV	CMAB73355/MT063996/ BtCoV/H.ruber/CMR/ CMAB73355r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	95% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB73357/MT063997/ BtCoV/H.ruber/CMR/ CMAB73357r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	95% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB73364/MT063985/ BtCoV/H.ruber/CMR/ CMAB73364r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	95% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB73372/MT063984/ BtCoV/H.ruber/CMR/ CMAB73372r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	95% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	CMAB73376/MT064047/BtCoV/H.ruber/CMR/CMAB73376o/2017	*Hipposideros ruber*/oral swab	11 April 2017/South	95% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB73401/MT063999/BtCoV/H.ruber/CMR/CMAB73401r/2017	*Hipposideros ruber*/rectal swab	14 April 2017/South	95% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB75014/MT064026/BtCoV/H.ruber/CMR/CMAB75014r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	95% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB75051/MT064035/BtCoV/H.ruber/CMR/CMAB75051r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	94% Human coronavirus 229E PREDICT_OTBA41-20,130,602 (KX285803)
Bat CoV cluster 1 D (Q)/Alpha CoV	CMAB72622/MT063978/BtCoV/H.ruber/CMR/CMAB72622r/2018	*Hipposideros ruber*/rectal swab	18 February 2017/South	97% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB75013/MT064024/BtCoV/H.ruber/CMR/CMAB75013o/2018	*Hipposideros ruber*/oral swab	30 May 2018/South	98% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB75013/MT064025/BtCoV/H.ruber/CMR/CMAB75013r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	98% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB75019/MT064028/BtCoV/H.ruber/CMR/CMAB75019r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	96% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB75026/MT064029/BtCoV/H.ruber/CMR/CMAB75026r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	98% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
	CMAB75030 /MT064031/BtCoV/H.ruber/CMR/CMAB75030r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	97% Rousettus aegyptiacus bat coronavirus 229E-related isolate 5425 (MN611517)
Bat CoV cluster 1 E (Q)/Alpha CoV	CMAB73368/MT064018/BtCoV/H.ruber/CMR/CMAB73368o/2017	*Hipposideros ruber*/oral swab	11 April 2017/South	94% Camel alphacoronavirus Camel229E isolate Camel229E-CoV/KCSP1/KEN/2015 (KU291449)
	CMAB73368/MT063980/BtCoV/H.ruber/CMR/CMAB73368r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	94% Camel alphacoronavirus Camel229E isolate Camel229E-CoV/KCSP1/KEN/2015 (KU291449)
	CMAB74746/MT064013/BtCoV/H.ruber/CMR/CMAB74746r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	94% 229E-related bat coronavirus isolate BtCoV/FO1A-F2/Hip_aba/GHA/2010 (KT253270)
	CMAB74747/MT064014/BtCoV/H.ruber/CMR/CMAB74747r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	94% 229E-related bat coronavirus isolate BtCoV/FO1A-F2/Hip_aba/GHA/2010 (KT253270)
Bat CoV cluster 1 F (Q)/Alpha CoV	CMAB75005/MT064022/BtCoV/H.curtus/CMR/CMAB75005r/2018	*Hipposideros curtus*/rectal swab	30 May 2018/South	94% 229E-related bat coronavirus strain BtKY229E-1 (KY073747)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
Bat CoV cluster 2 A (W)/Beta	CMAB72008/MT081995/ BtCoV/H.ruber/CMR/ CMAB72008r/2016	*Hipposideros ruber*/rectal swab	13 September 2016/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72017/MT082013/ BtCoV/H.ruber/CMR/ CMAB72017r/2016	*Hipposideros ruber*/rectal swab	13 September 2016/South	89% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72021/MT081992/ BtCoV/H.ruber/CMR/ CMAB72021r/2016	*Hipposideros ruber*/rectal swab	13 September 2016/South	89% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72044/MT082014/ BtCoV/H.ruber/CMR/ CMAB72044r/2016	*Hipposideros ruber*/rectal swab	13 September 2016/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72132/MT082028/ BtCoV/H.ruber/CMR/ CMAB72132o/2016	*Hipposideros ruber*/oral swab	29 October 2016/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72132/MT082052/ BtCoV/H.ruber/CMR/ CMAB72132r/2016	*Hipposideros ruber*/rectal swab	29 October 2016/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72135/MT082015/ BtCoV/H.ruber/CMR/ CMAB72135r/2016	*Hipposideros ruber*/rectal swab	29 October 2016/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72190/MT082025/ BtCoV/H.ruber/CMR/ CMAB72190r/2016	*Hipposideros ruber*/rectal swab	1 November 2016/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72211/MT082011/ BtCoV/H.ruber/CMR/ CMAB72211r/2016	*Hipposideros ruber*/rectal swab	1 November 2016/South	92% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72217/MT082022/ BtCoV/H.ruber/CMR/ CMAB72217r/2016	*Hipposideros ruber*/rectal swab	1 November 2016/South	92% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB72621/MT082017/ BtCoV/H.ruber/CMR/ CMAB72621r/2017	*Hipposideros ruber*/rectal swab	18 February 2017/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB73361/MT082005/ BtCoV/H.ruber/CMR/ CMAB73361r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB73371/MT082027/ BtCoV/H.ruber/CMR/ CMAB73371r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB74557/MT082075/ BtCoV/H.fuliginosus/CMR/ CMAB74557o/2017	*Hipposideros fuliginosus*/oral swab	19 December 2017/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB74578/MT082074/ BtCoV/H.ruber/CMR/ CMAB74578r/2017	*Hipposideros ruber*/rectal swab	19 December 2017/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB74787/MT082076/ BtCoV/H.ruber/CMR/ CMAB74787r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	89% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
Bat CoV cluster 2 B (W)/Beta	CMAB75012/MT082083/ BtCoV/H.ruber/CMR/ CMAB75012/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB75017/MT082095/ BtCoV/H.ruber/CMR/ CMAB75017o/2018	*Hipposideros ruber*/oral swab	30 May 2018/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB75017/MT082096/ BtCoV/H.ruber/CMR/ CMAB75017r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)
	CMAB75043/MT082122/ BtCoV/H.ruber/CMR/ CMAB75043r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	90% Bat SARS-like coronavirus isolate BtCoV-Zim035Mag (MG000872)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	GVF-CM-ECO70005/ KX284977/BtCoV/ M.condylurus/CMR/ ECO70005r/2013	*Mops condylurus*/rectal swab	1 March 2013/East	98% Bat coronavirus isolate 19,207 (MN183181)
Bat CoV cluster 2 C (W)/Beta	CMAB75012/MT064023/ BtCoV/H.ruber/CMR/ CMAB75012r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	84% Coronavirus PREDICT CoV-20 PREDICT_CoV-20/ZB12062 (KX286249)
	CMAB75042/MT064032/ BtCoV/H.ruber/CMR/ CMAB75042r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	86% Coronavirus PREDICT CoV-62 PREDICT_CoV-62/AATKH (KX285866)
	CMAB75043/MT064033/ BtCoV/H.ruber/CMR/ CMAB75043r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	83% Coronavirus PREDICT CoV-20 PREDICT_CoV-20/ZB12062 (KX286249)
Bat CoV cluster 2 D (W)/Beta	CMAB72190/MT063979/ BtCoV/H.ruber/CMR/ CMAB72190r/2016	*Hipposideros ruber*/rectal swab	1 November 2016/South	83% Coronavirus PREDICT CoV-62 PREDICT_CoV-62/AATKH (KX285866)
	CMAB73371/MT063994/ BtCoV/H.ruber/CMR/ CMAB73371r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	82% Coronavirus PREDICT CoV-20 PREDICT_CoV-20/ZB12062 (KX286249)
Bat CoV cluster 3 (W)/Beta	CMAB74992/MT082087/ BtCoV/R.aegyptiacus/ CMR/CMAB74992r/2018	*Rousettus aegyptiacus*/rectal swab	13 May 2018/South	100% Bat coronavirus isolate CMR66 (MG693170)
	CMAB74998/MT082088/ BtCoV/R.aegyptiacus/ CMR/CMAB74998r/2018	*Rousettus aegyptiacus*/rectal swab	28 May 2018/South	99% Bat coronavirus isolate CMR66 (MG693170)
	CMAB74999/MT082089/ BtCoV/R.aegyptiacus/ CMR/CMAB74999r/2018	*Rousettus aegyptiacus*/rectal swab	28 May 2018/South	99% Bat coronavirus isolate CMR66 (MG693170)
	GVF-CM-ECO06464/ KX284959/BtCoV/ R.aegyptiacus/CMRECO 06464r/2013	*Rousettus aegyptiacus*/rectal swab	29 January 2013/Center	99% Bat coronavirus iso-late BatCoV03/KEN/Kwale (MH170074)
	GVF-CM-ECO06646/ KX284961/BtCoV/E.helvum/ CMR/ECO06646r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	99% Bat coronavirus iso-late BatCoV03/KEN/Kwale (MH170074)
Bat CoV cluster 3 (Q)/Beta	CMAB74998/MT064020/ BtCoV/R.aegyptiacus/ CMR/CMAB74998r/2018	*Rousettus aegyptiacus*/rectal swab	28 May 2018/South	98% Bat coronavirus isolate CMR66 (MG693170)
	CMAB75003/MT064021/ BtCoV/R.aegyptiacus/ CMR/CMAB75003r/2018	*Rousettus aegyptiacus*/rectal swab	28 May 2018/South	99% Bat coronavirus isolate CMR66 (MG693170)
Bat CoV cluster 4 A (Q)	CMAB72188/MT063975/ BtCoV/H.ruber/CMR/ CMAB72188r/201	*Hipposideros ruber*/rectal swab	1 November 2016/South	92% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB73356/MT063972/ BtCoV/H.ruber/CMR/ CMAB73356r/2017	*Hipposideros ruber*/rectal swab	11 April 2017/South	92% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB74581/MT064009/ BtCoV/H.ruber/CMR/ CMAB74581r/2017	*Hipposideros ruber*/rectal swab	19 December 2017/South	93% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB74730/MT064010/ BtCoV/H.ruber/CMR/ CMAB74730r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	93% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB74742/MT064012/ BtCoV/H.ruber/CMR/ CMAB74742r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	93% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB74751/MT064011/ BtCoV/H.ruber/CMR/ CMAB74751r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	93% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB74759/MT064019/ BtCoV/H.ruber/CMR/ CMAB74759r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	93% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)

(continued)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
Bat CoV cluster 4 B (Q)/Beta	CMAB72610/MT063977/ BtCoV/H.ruber/CMR/ CMAB72610r/2017	*Hipposideros ruber*/rectal swab	18 February 2017/South	92% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB75015/MT064027/ BtCoV/H.ruber/CMR/ CMAB75015r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	93% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB75028/MT064030/ BtCoV/H.ruber/CMR/ CMAB75028r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	93% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
	CMAB75048/MT064034/ BtCoV/H.ruber/CMR/ CMAB75048r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	92% Coronavirus PREDICT CoV-44 PREDICT_CoV-44/AABRY (KX286327)
Bat CoV cluster 4 C (W)/Beta	CMAB74742/MT081974/ BtCoV/H.ruber/CMR/ CMAB74742r/2018	*Hipposideros ruber*/rectal swab	19 January 2018/South	98% Bat coronavirus Gabon/292/2009 (JX174638)
	CMAB75015/MT081975/ BtCoV/H.ruber/CMR/ CMAB75015r/2018	*Hipposideros ruber*/rectal swab	30 May 2018/South	98% Bat coronavirus Gabon/292/2009 (JX174638)
Bat CoV cluster 5 A (Q)/Beta	CMAB73538/MT063973/ BtCoV/M.gigas/CMR/ CMAB73538r/2017	*Macronycteris gigas*/rectal swab	9 June 2017/South	96% Zaria bat coronavirus strain ZBCoV (HQ166910)
	CMAB73542/MT063971/ BtCoV/M.gigas/CMR/ CMAB73542r/2017	*Macronycteris gigas*/rectal swab	9 June 2017/South	97% Zaria bat coronavirus strain ZBCoV (HQ166910)
	CMAB73545/MT063970/ BtCoV/M.gigas/CMR/ CMAB73545r/2017	*Macronycteris gigas*/rectal swab	9 June 2017/South	97% Zaria bat coronavirus strain ZBCoV (HQ166910)
	CMAB73578/MT064003/ BtCoV/M.gigas/CMR/ CMAB73578o/2017	*Macronycteris gigas*/oral swab	9 June 2017/South	96% Zaria bat coronavirus strain ZBCoV (HQ166910)
Bat CoV cluster 5 B (Q)/Beta	CMAB72452/MT063976/ BtCoV/M.gigas/CMR/ CMAB72452r/2017	*Macronycteris gigas*/rectal swab	15 January 2017/South	94% Zaria bat coronavirus strain ZBCoV (HQ166910)
	CMAB73546/MT063998/ BtCoV/M.gigas/CMR/ CMAB73546r/2017	*Macronycteris gigas*/rectal swab	9 June 2017/South	94% Zaria bat coronavirus strain ZBCoV (HQ166910)
	CMAB73578/MT063969/ BtCoV/M.gigas/CMR/ CMAB73578r/2017	*Macronycteris gigas*/rectal swab	9 June 2017/South	95% Zaria bat coronavirus strain ZBCoV (HQ166910)
Bat CoV cluster 5 C (W)/Beta	CMAB73578/MT082024/ BtCoV/M.gigas/CMR/ CMAB73578r/2017	*Macronycteris gigas*/rectal swab	9 June 2017/South	95% Zaria bat coronavirus strain ZBCoV (HQ166910)
Bat CoV cluster 5 D (W)/Beta	CMAB73546/MT082054/ BtCoV/M.gigas/CMR/ CMAB73546r/2017	*Macronycteris gigas*/rectal swab	9 June 2017/South	98% Bat coronavirus isolate 13GB0273 (MG963188)
Bat CoV cluster 5 E (Q)/Beta	CMAB72450/MT063995/ BtCoV/M.gigas/CMR/ CMAB72450o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	94% Zaria bat coronavirus strain ZBCoV (HQ166910)
Bat CoV cluster 6 (W)/Alpha	CMAB74957/MT082121/ BtCoV/R.alcyone/CMR/ CMAB74957r/2018	*Rhinolophus* cf. alcyone/rectal swab	8 May 2018/South	92% Kenya bat coronavirus BtKY83 (GU065427)
Bat CoV cluster 6 (Q)/Alpha	CMAB74957/MT064051/ BtCoV/R.alcyone/CMR/ CMAB73578r/2017	*Rhinolophus* cf. alcyone/rectal swab	8 May 2018/South	91% Coronavirus PREDICT CoV-70 PREDICT_CoV-70/OTBA29-20,130,601 (KX285812)
Bat CoV cluster 7 (W)/Beta	GVF-CM-ECO05710/ KX284951/BtCoV/ M.pusillus/CMR/ ECO05710o/2010	*Micropteropus pusillus*/oral swab	6 June 2010/Southwest	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)

(continued)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	GVF-CM-ECO06214/ KX284954/BtCoV/ M.pusillus/CMR/ ECO06214o/2011	*Micropteropus pusillus*/oral swab	10 June 2011/Center	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO06409/ KX284957/BtCoV/ E.franqueti/CMR/ ECO06409r/2013	*Epomops franqueti*/rectal swab	14 January 2013/North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO06417/ KX284958/BtCoV/ E.franqueti/CMR/ ECO06417r/2013	*Epomops franqueti*/rectal swab	14 January 2013/North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70284/ KX284985/BtCoV/ M.pusillus/CMR/ ECO70284o/2013	*Micropteropus pusillus*/oral swab	24 April 2013/Center	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70332/ KX284987/BtCoV/ M.pusillus/CMR/ ECO70332o/2013	*Micropteropus pusillus*/oral swab	30 May 2013/Southwest	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70332/ KX284986/BtCoV/ M.pusillus/CMR/ ECO70332r/2013	*Micropteropus pusillus*/rectal swab	30 May 2013/Southwest	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70379/ KX284990/BtCoV/ M.pusillus/CMR/ ECO70379o/2013	*Micropteropus pusillus*/oral swab	30 May 2013/Southwest	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70379/ KX284989/BtCoV/ M.pusillus/CMR/ ECO70379r/2013	*Micropteropus pusillus*/rectal swab	30 May 2013/Southwest	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70509/ KX284994/BtCoV/ E.gambianus/CMR/ ECO70509o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70509/ KX284993/BtCoV/ E.gambianus/CMR/ ECO70509r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70514/ KX284998/BtCoV/ E.gambianus/CMR/ ECO70514o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70514/ KX284999/BtCoV/ E.gambianus/CMR/ ECO70514r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70516/ KX285000/BtCoV/ S.leucogaster/CMR/ ECO70516o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70516/ KX285001/BtCoV/ S.leucogaster/CMR/ ECO70516r/2013	*Scotophilus leucogaster*/rectal swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70521/ KX285007/BtCoV/ E.gambianus/CMR/ ECO70521o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	98% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70521/ KX285006/BtCoV/ E.gambianus/CMR/ ECO70521r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	98% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	GVF-CM-ECO70527/ KX285009/BtCoV/ E.gambianus/CMR/ ECO70527o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70527/ KX285008/BtCoV/ E.gambianus/CMR/ ECO70527r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70536/ KX285013/BtCoV/ E.gambianus/CMR/ ECO70536o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70536/ KX285012/BtCoV/ E.gambianus/CMR/ ECO70536r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70591/ KX285023/BtCoV/ E.gambianus/CMR/ ECO70591r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70592/ KX285024/BtCoV/ E.gambianus/CMR/ ECO70592o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70592/ KX285025/BtCoV/ E.gambianus/CMR/ ECO70592r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70594/ MT221714/BtCoV/ E.gambianus/CMR/ ECO70594o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70597/ KX285027/BtCoV/ E.gambianus/CMR/ ECO70597o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70597/ KX285028/BtCoV/ E.gambianus/CMR/ ECO70597r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
	GVF-CM-ECO70598/ KX285029/BtCoV/ E.gambianus/CMR/ ECO70598o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Kenya bat coronavirus/BtKY56/BtKY55 PREDICT-GVF-RC-1006 (KX285501)
Bat CoV cluster 8 (W)/Alpha	GVF-CM-ECO00122/ KX284945/BtCoV/ E.helvum/CMR/ ECO00122p/2004	*Eidolon helvum*/plasma	8 May 2004/Center	98% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO00159/ KX284946/BtCoV/ E.helvum/CMR/ ECO00159p/2004	*Eidolon helvum*/plasma	8 May 2004/Center	98% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO05521/ MT221711/BtCoV/ M.woermanni/CMR/ ECO05521s/2010	*Megaloglossus woermanni*/spleen	5 January 2010/East	100% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO05521/ KX284948/BtCoV/ M.woermanni/CMR/ ECO05521l/2010	*Megaloglossus woermanni*/liver	5 January 2010/East	99% Bat coronavirus isolate 19,207 (MN183181)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	GVF-CM-ECO05817/ KX284952/BtCoV/ M.pusillus/CMR/ ECO05817l/2010	*Micropteropus pusillus*/liver	10 October 2010/Center	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70001/ KX284974/BtCoV/ M.condylurus/CMR/ ECO70001r/2013	*Mops condylurus*/rectal swab	1 March 2013/East	98% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70002/ KX284975/BtCoV/ M.condylurus/CMR/ ECO70002o/2013	*Mops condylurus*/oral swab	1 March 2013/East	98% Chaerephon bat coronavirus/Kenya/KY22/2006 PREDICT-AATCA (KX285352)
	GVF-CM-ECO70002/ KX284976/BtCoV/ M.condylurus/CMR/ ECO70002r/2013	*Mops condylurus*/rectal swab	1 March 2013/East	98% Chaerephon bat coronavirus/Kenya/KY22/2006 PREDICT-AAOSV (KX285262)
	GVF-CM-ECO70010/ KX284978/BtCoV/ M.condylurus/CMR/ ECO70010o/2013	*Mops condylurus*/oral swab	1 March 2013/East	98% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70011/ KX284979/BtCoV/ M.condylurus/CMR/ ECO70011o/2013	*Mops condylurus*/oral swab	1 March 2013/East	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70023/ KX284980/BtCoV/ M.condylurus/CMR/ ECO70023r/2013	*Mops condylurus*/rectal swab	1 March 2013/East	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70102/ KX284983/BtCoV/ M.condylurus/CMR/ ECO70102o/2013	*Mops condylurus*/oral swab	1 March 2013/Littoral	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70102/ KX284982/BtCoV/ M.condylurus/CMR/ ECO70102r/2013	*Mops condylurus*/rectal swab	1 March 2013/Littoral	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70511/ KX284995/BtCoV/ E.gambianus/CMR/ ECO70511o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70511/ KX284996/BtCoV/ E.gambianus/CMR/ ECO70511r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70520/ KX285005/BtCoV/ P.inexspectatus/CMR/ ECO70520o/2013	*Pipistrellus inexspectatus*/oral swab	4 July 2013/Far North	98% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70520/ KX285004/BtCoV/ P.inexspectatus/CMR/ ECO70520r/2013	*Pipistrellus inexspectatus*/rectal swab	4 July 2013/Far North	98% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70528/ KX285011/BtCoV/ E.gambianus/CMR/ ECO70528o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70528/ KX285010/BtCoV/ E.gambianus/CMR/ ECO70528r/2013	*Epomophorus gambianus*/rectal swab	4 July 2013/Far North	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70538/ KX285014/BtCoV/ M.condylurus/CMR/ ECO70538o/2013	*Mops condylurus*/oral swab	4 July 2013/Far North	99% Bat coronavirus isolate 19,207 (MN183181)

(continued)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	GVF-CM-ECO70543/ MT221718/BtCoV/ M.condylurus/CMR/ ECO70543o/2013	*Mops condylurus*/oral swab	4 July 2013/Far North	97% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70546/ KX285015/BtCoV/ M.condylurus/CMR/ ECO70546r/2013	*Mops condylurus*/rectal swab	4 July 2013/Far North	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70559/ KX285017/BtCoV/ M.condylurus/CMR/ ECO70559r/2013	*Mops condylurus*/rectal swab	4 July 2013/Far North	98% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70565/ KX285018/BtCoV/ M.condylurus/CMR/ ECO70565o/2013	*Mops condylurus*/oral swab	4 July 2013/Far North	98% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70565/ KX285019/BtCoV/ M.condylurus/CMR/ ECO70565r/2013	*Mops condylurus*/rectal swab	4 July 2013/Far North	98% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70594/ KX285026/BtCoV/ E.gambianus/CMR/ ECO70594o/2013	*Epomophorus gambianus*/oral swab	4 July 2013/Far North	99% Bat coronavirus isolate 19,207 (MN183181)
	GVF-CM-ECO70614/ KX285033/BtCoV/ S.leucogaster/CMR/ ECO70614o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/North	99% Bat coronavirus isolate 19,207 (MN183181)
Bat CoV cluster 9 (W)/Beta	CMAB73427/MT082007/ BtCoV/E.helvum/CMR/ CMAB73427r/2017	*Eidolon helvum*/rectal swab	29 May 2017/Far North	100% Bat coronavirus isolate CMR705-P13 (MG693172)
	GVF-CM-ECO06464/ KX284960/BtCoV/ R.aegyptiacus/CMR/ ECO06464r/2013	*Rousettus aegyptiacus*/rectal swab	29 January 2013/Center	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AAUEY (KX285360)
	GVF-CM-ECO06646/ KX284962/BtCoV/ E.helvum/CMR/ ECO06646r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AAUEY (KX285360)
	GVF-CM-ECO06648/ KX284963/BtCoV/ E.helvum/CMR/ ECO06648r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	99% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATLD (KX285370)
	GVF-CM-ECO06648/ MT221713/BtCoV/ E.helvum/CMR/ ECO06648r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	100% Bat coronavirus isolate KSA282 (MH396479)
	GVF-CM-ECO06653/ KX284964/BtCoV/ E.helvum/CMR/ ECO06653r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	100% Bat coronavirus isolate CMR705-P13 (MG693172)
	GVF-CM-ECO06655/ KX284965/BtCoV/ E.helvum/CMR/ ECO06655r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	99% Kenya bat coronavirus BtKY88 (GU065432)
	GVF-CM-ECO06656/ KX284966/BtCoV/ E.helvum/CMR/ ECO06656o/2012	*Eidolon helvum*/oral swab	9 September 2012/Center	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATJM (KX285342)
	GVF-CM-ECO06659/ KX284967/BtCoV/ E.helvum/CMR/ ECO06659o/2012	*Eidolon helvum*/oral swab	9 September 2012/Center	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATEU (KX285263)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	GVF-CM-ECO06659/ KX284968/BtCoV/ E.helvum/CMR/ ECO06659r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATEU (KX285263)
	GVF-CM-ECO06661/ KX284969/BtCoV/ E.helvum/CMR/ ECO06661o/2012	*Eidolon helvum*/oral swab	9 September 2012/Center	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATJS (KX285347)
	GVF-CM-ECO06661/ KX284970/BtCoV/ E.helvum/CMR/ ECO06661r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATJS (KX285347)
	GVF-CM-ECO06662/ KX284971/BtCoV/ E.helvum/CMR/ ECO06662r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	99% Bat coronavirus isolate KSA299 (MH396477)
	GVF-CM-ECO06663/ KX284973/BtCoV/ E.helvum/CMR/ ECO06663o/2012	*Eidolon helvum*/oral swab	9 September 2012/Center	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATIH (KX285314)
	GVF-CM-ECO06663/ KX284972/BtCoV/ E.helvum/CMR/ ECO06663r/2012	*Eidolon helvum*/rectal swab	9 September 2012/Center	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATIH (KX285314)
	GVF-CM-ECO70519/ KX285002/BtCoV/ S.dinganii/CMR/ ECO70519o/2013	*Scotophilus dinganii*/oral swab	4 July 2013/Far North	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATJM (KX285342)
	GVF-CM-ECO70519/ KX285003/BtCoV/ S.dinganii/CMR/ ECO70519r/2013	*Scotophilus dinganii*/rectal swab	4 July 2013/Far North	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATJM (KX285342)
	GVF-CM-ECO70566/ KX285020/BtCoV/ M.conylurus/CMR/ ECO70566r/2013	*Mops condylurus*/rectal swab	4 July 2013/Far North	100% Eidolon bat coronavirus/Kenya/KY24/2006 PREDICT-AATJM (KX285342)
Bat CoV cluster 10 (W)/Alpha	CMAB71074/MT221707/ BtCoV/E.franqueti/CMR/ CMAB71074o/2015	*Epomops franqueti*/oral swab	12 August 2015/South	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	CMAB71074/MT221708/ BtCoV/E.franqueti/CMR/ CMAB71074r/2015	*Epomops franqueti*/rectal swab	12 August 2015/South	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	CMAB74987/MT082082/ BtCoV/S.nux/CMR/ CMAB74987r/2018	*Scotophilus nux*/rectal swab	13 May 2018/South	99% Bat alphacoronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70504/ KX284992/BtCoV/ S.leucogaster/CMR/ ECO70504o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/Far North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70504/ KX284991/BtCoV/ S.leucogaster/CMR/ ECO70504r/2013	*Scotophilus leucogaster*/rectal swab	4 July 2013/Far North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	GVF-CM-ECO70512/ KX284997/BtCoV/ S.leucogaster/CMR/ ECO70512o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/Far North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70512/ MT221716/BtCoV/ S.leucogaster/CMR/ ECO70512o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/Far North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70569/ KX285021/BtCoV/ M.condylurus/CMR/ ECO70569o/2013	*Mops condylurus*/oral swab	4 July 2013/Far North	99% Bat alphacoronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70578/ KX285022/BtCoV/ S.leucogaster/CMR/ ECO70578o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/Far North	99% Bat alphacoronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70601/ KX285030/BtCoV/ S.dinganii/CMR/ ECO70601r/2013	*Scotophilus dinganii*/rectal swab	4 July 2013/Far North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70608/ KX285031/BtCoV/ S.leucogaster/CMR/ ECO70608r/2013	*Scotophilus leucogaster*/rectal swab	4 July 2013/North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70611/ KX285032/BtCoV/ S.leucogaster/CMR/ ECO70611o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70615/ KX285034/BtCoV/ S.leucogaster/CMR/ ECO70615o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70616/ KX285035/BtCoV/ S.leucogaster/CMR/ ECO70616r/2013	*Scotophilus leucogaster*/rectal swab	4 July 2013/North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70618/ MT221719/BtCoV/ S.leucogaster/CMR/ ECO70618o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/North	99% Bat alphacoronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70622/ MT221715/BtCoV/ S.leucogaster/CMR/ ECO70622o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/North	100% Bat alpha-coronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
	GVF-CM-ECO70623/ MT221717/BtCoV/ S.leucogaster/CMR/ ECO70623o/2013	*Scotophilus leucogaster*/oral swab	4 July 2013/North	99% Bat alphacoronavirus strain BtCoV/20160411_DC68/ Scotophilus/RSA (MG193606)
Bat CoV cluster 11 A (Q)/Alpha	CMAB72456/MT063986/ BtCoV/M.gigas/CMR/ CMAB72456o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	88% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	CMAB72457/MT064005/ BtCoV/M.gigas/CMR/ CMAB72457o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	89% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB72459/MT064001/ BtCoV/M.gigas/CMR/ CMAB72459o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	88% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB72461/MT063982/ BtCoV/M.gigas/CMR/ CMAB72461o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	87% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB72463/MT063981/ BtCoV/M.gigas/CMR/ CMAB72463o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	88% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB72464/MT064002/ BtCoV/M.gigas/CMR/ CMAB72464o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	88% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB72465/MT063983/ BtCoV/M.gigas/CMR/ CMAB72465o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	89% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB72467/MT064006/ BtCoV/M.gigas/CMR/ CMAB72467o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	88% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB73459/MT064007/ BtCoV/M.gigas/CMR/ CMAB73459o/2017	*Macronycteris gigas*/oral swab	9 June 2017/South	88% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB73539/MT064048/ BtCoV/M.gigas/CMR/ CMAB73539o/2017	*Macronycteris gigas*/oral swab	9 June 2017/South	87% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB73549/MT064008/ BtCoV/M.gigas/CMR/ CMAB73549o/2017	*Macronycteris gigas*/oral swab	9 June 2017/South	87% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB73551/MT064049/ BtCoV/M.gigas/CMR/ CMAB73551o/2017	*Macronycteris gigas*/oral swab	9 June 2017/South	87% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
	CMAB73554/MT064004/ BtCoV/M.gigas/CMR/ CMAB73554o/2017	*Macronycteris gigas*/oral swab	9 June 2017/South	87% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
Bat CoV cluster 11 B (Q)/Alpha	CMAB72455/MT064000/ BtCoV/M.gigas/CMR/ CMAB72455o/2017	*Macronycteris gigas*/oral swab	15 January 2017/South	88% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
Bat CoV cluster 11 C (Q)/Alpha	CMAB72449/MT063974/ BtCoV/M.gigas/CMR/ CMAB72449r/2017	*Macronycteris gigas*/rectal swab	15 January 2017/South	87% Coronavirus PREDICT CoV-54 PREDICT_CoV-54/GVF-RC-1049 (KX286263)
Bat CoV cluster 12 (W)/Beta	CMAB71162/MT081985/ BtCoV/M.woemanni/CMR/ CMAB71162r/2015	*Megaloglossus woermanni*/rectal swab	29 September 2015/South	97% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
	CMAB74975/MT082086/ BtCoV/M.woemanni/CMR/ CMAB74975o/2018	*Megaloglossus woermanni*/oral swab	13 May 2018/South	97% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)

Table 2. (Continued)

Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
	GVF-CM-ECO05689/ KX284949/BtCoV/ R.aegyptiacus/ CMR/ECO05689s/2010	*Rousettus aegyptiacus*/spleen	5 June 2010/Southwest	99% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
	GVF-CM-ECO05689/ KX284950/BtCoV/ R.aegyptiacus/CMR/ ECO05689l/2010	*Rousettus aegyptiacus*/liver	5 June 2010/Southwest	97% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
	GVF-CM-ECO05852/ KX284953/BtCoV/ E.franqueti/CMR/ ECO05852s/2010	*Epomops franqueti*/spleen	23 October 2010/South	99% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
	GVF-CM-ECO06324/ KX284956/BtCoV/ M.woemanni/CMR/ ECO76324o/2012	*Megaloglossus woermanni*/oral swab	14 December 2012/Littoral	99% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
	GVF-CM-ECO70191/ KX284984/BtCoV/ M.woemanni/CMR/ ECO70191r/2013	*Megaloglossus woermanni*/rectal swab	10 April 2013/South	99% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
	GVF-CM-ECO70354/ KX284988/BtCoV/ M.woemanni/CMR/ ECO70354r/2013	*Megaloglossus woermanni*/rectal swab	30 May 2013/South west	97% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
	GVF-CM-ECO70554/ KX285016/BtCoV/ M.condylurus/CMR/ ECO70554r/2013	*Mops condylurus*/rectal swab	4 July 2013/Far North	99% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
	GVF-CM-ECO70775/ KX285036/BtCoV/ M.woemanni/CMR/ ECO70775r/2014	*Megaloglossus woermanni*/rectal swab	18 February 2014/South	99% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
	GVF-CM-ECO70788 /KX285037/BtCoV/ M.woemanni/CMR/ ECO70788o/2014	*Megaloglossus woermanni*/oral swab	18 February 2014/South	99% Coronavirus PREDICT CoV-30 PREDICT_CoV-30/CD115847 (KX285072)
Bat CoV cluster 13 (W)/Beta	CMAB71193/MT081984/ BtCoV/M.torquata/CMR/ CMAB71193o/2015	*Myonycteris torquata*/oral swab	29 September 2015/South	99% Coronavirus PREDICT CoV-66 PREDICT_CoV-66/130512Bt05 (KX285426)
	CMAB71193/MT081986/ BtCoV/M.torquata/CMR/ CMAB71193r/2015	*Myonycteris torquata*/rectal swab	29 September 2015/South	99% Coronavirus PREDICT CoV-66 PREDICT_CoV-66/130512Bt05 (KX285426)
Bat CoV cluster 14 (W)/Beta	GVF-CM-ECO70036/ KX284981/BtCoV/ H.caffer/CMR/ECO70036r/ 2013	*Hipposideros caffer*/rectal swab	1 March 2013/Center	90% Bat coronavirus Gabon/292/2009 (JX174638)
Bat CoV cluster 15 (W)/Alpha	GVF-CM-ECO05172/ KX284947/BtCoV/S.nux/ CMR/ECO05172r/2009	*Scotophilus nux*/rectal swab	11 January 2009/South	82% Alphacoronavirus sp. isolate SPA_EPI5_Myomyo_ Minsch61_8E_p25 (KY423464)
Civet CoV cluster 16 (W)/Alpha	CMAV71560/MT221709/ Civet-CoV/N.binotata/ CMR/CMAV71560r/2016	*Nandinia binotata*/rectal swab	31 May 2016/South	87% Canine coronavirus strain 1-71 (JQ404409)
Bat CoV cluster 17 (Q)/Alpha	CMAB74957/MT064052/ BtCoV/R.alcyone/CMR/ CMAB74957r/2018	*Rhinolophus* cf. alcyone/rectal swab	8 May 2018/South	88% Coronavirus PREDICT CoV-65 PREDICT_CoV-65/OTBA07-20,130,531 (KX285807)
Bat CoV cluster 18 (Q)/Alpha	CMAB75000/MT064017/ BtCoV/R.aegyptiacus/CMR/ CMAB75000o/2018	*Rousettus aegyptiacus*/oral swab	28 May 2018/South	83% Bat coronavirus BtCoV/Rh/YN2012 isolate BtCoV/Rh/YN2012_Ra13591 (MG916904)

(continued)

Table 2. (Continued)

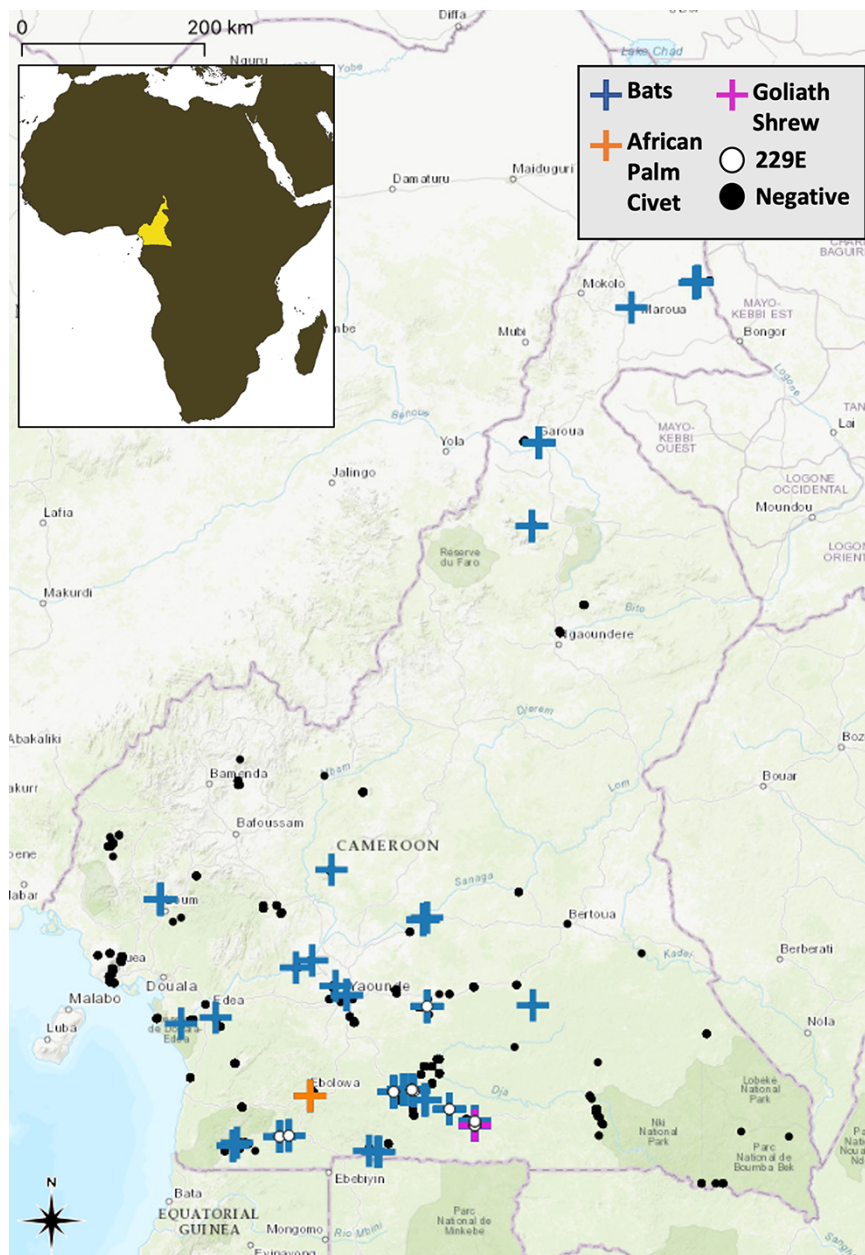| Cluster/genus	Animal ID/GenBank ID/virus name	Host species/sample type	Sample date/region	BLAST 6 April 2020
Shrew CoV cluster 19 (Q)/Alpha	CMAR74882/MT064045/Shrew-CoV/C.goliath/CMR/CMAR74882r/2018	*Crocidura goliath*/rectal swab	10 May 2018/South	84% Wencheng Sm shrew coronavirus isolate Ruian-90 (KY967725)

Figure 1. Sampling map: Map of Cameroon highlighting where samples were collected.

Bat sampling was conducted in many areas of the country, but 46.7 per cent of the bats were sampled in the South Region (Supplement 4). Male bats were slightly overrepresented (53.5 per cent) compared to females; however, female bats were significantly (P < 0.01) more likely to have a positive CoV test (8.2 per cent) than male bats (5.6 per cent). Differences in CoV RNA-positive rate were also observed between adult (7.4 per cent, $n = 1495$) and younger bats (10.3 per cent, $n = 87$), but these were not statistically significant. Samples collected at animal–human interfaces categorized as 'Tourism' (1.5 per cent, $n = 136$) and 'Value chain' (3.1 per cent, $n = 488$) were significantly less likely (P < 0.001) to be CoV RNA positive than those collected at 'other peridomestic' interfaces (8.1 per cent, $n = 1957$) such as in or near human dwellings and temporary settlements or close to crop production.

3.2 Civet CoV

The CoV RNA detected in an African palm civet *(Nandinia binotata)* resembles that of an alphacoronavirus and, on the nucleotide level (BLASTN), is most closely related to canine CoV, feline CoV, and porcine CoV (TGEV) with 87 per cent, 86 per cent, and 85 per cent identities, respectively (Table 2). Phylogenetic analysis places the RNA in a cluster with dog, cat, mink, and pig CoVs (Fig. 2).

3.3 Shrew CoV

The CoV RNA detected in a Goliath shrew *(Crocidura goliath)* resembles that of an alphacoronavirus and is on the nucleotide level (BLASTN) closest related to isolates of Wencheng Sm shrew CoV

(from a *Suncus murinus*) and isolates of Coronavirus PREDICT CoV-46 (from a *Crocidura* sp.), with up to 84 per cent and 83 per cent identities, respectively (Table 2). Phylogenetic analysis places the RNA in a cluster with other shrew CoVs as part of a basal branch of alphacoronaviruses (Fig. 3).

3.4 Bat CoVs

The CoV RNA detected in the 175 bats form 17 different genetic clusters, of which 8 coincide with alpha and 9 with beta CoVs. The majority of sequences share identities above 90 per cent with known bat CoVs, while the sequences in up to 6 of the 17 clusters do not (bat CoV clusters 2, 11, 14, 15, 17, and 18)
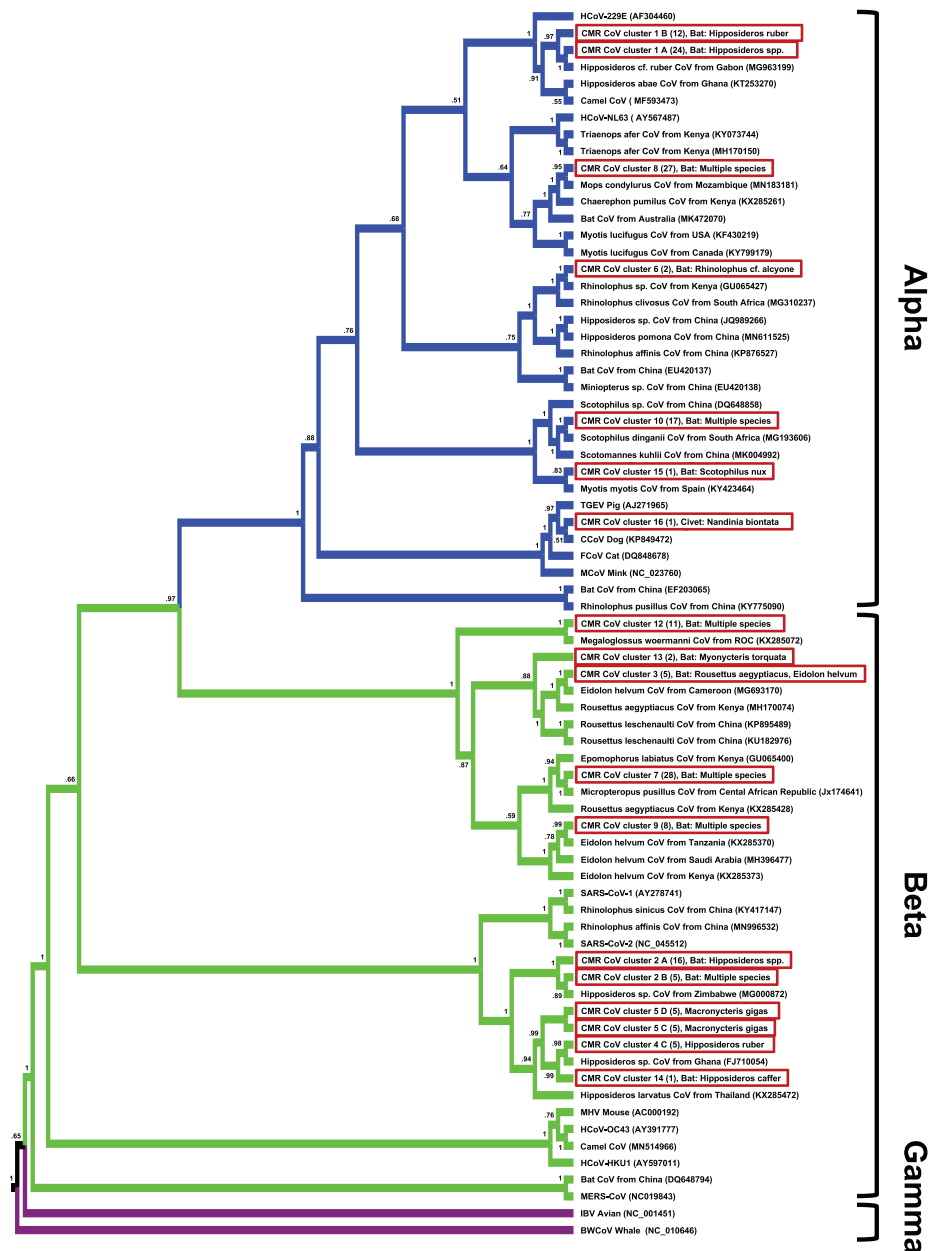
Figure 2. Phylogenetic tree: Maximum likelihood phylogenetic tree of coronavirus sequences presented as a proportional cladogram based on the RdRp region targeted by the PCR by Watanabe et. al. (Watanabe et al. 2010). The sequences detected during the project are highlighted by red boxes, and numbers in brackets indicate the number of sequences sharing more than 95 per cent nucleotide identities. GenBank accession numbers are listed for previously published sequences, while sequences obtained during the project are identified by cluster names (compare Table 2). Numbers at nodes indicate bootstrap support.
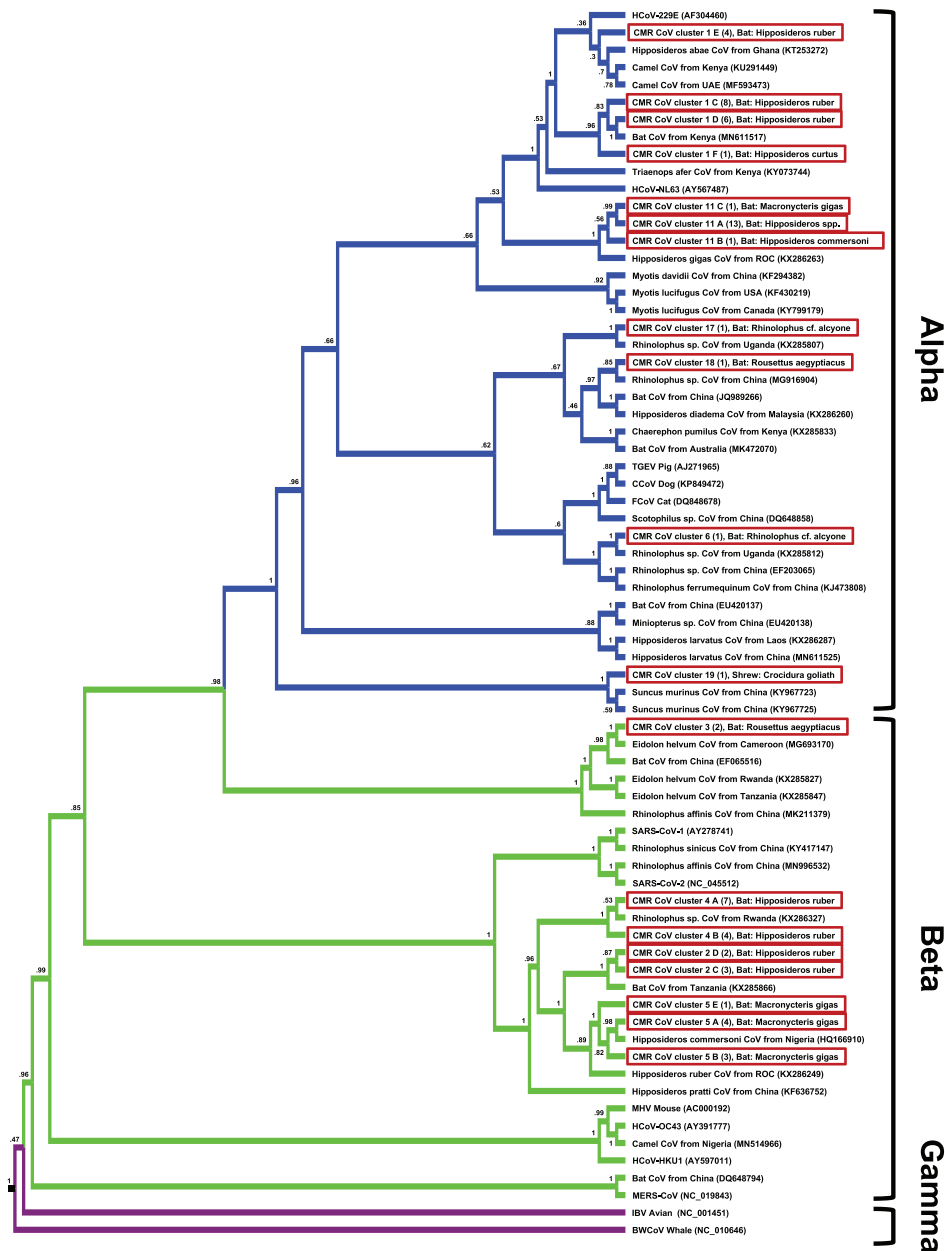
Figure 3. Phylogenetic tree: Maximum likelihood phylogenetic tree of coronavirus sequences presented as a proportional cladogram based on the RdRp region targeted by the PCR by Quan et. al. (Quan et al. 2010). The sequences detected during the project are highlighted by red boxes, and numbers in brackets indicate the number of sequences sharing more than 95 per cent nucleotide identities. GenBank accession numbers are listed for previously published sequences, while sequences obtained during the project are identified by cluster names (compare Table 2). Red boxes indicate isolates from this study. Numbers at nodes indicate bootstrap support.

(Table 2, Figs 2 and 3). In 170 bats, RNA corresponding to a single CoV was detected, with 89 resembling alpha CoVs and 81 beta CoVs. In five bats, RNA corresponding to two different CoVs was found. In two of these cases, RNA of both an alpha and a beta CoV was present; in two bats, the RNA of two different beta CoVs was detected; and in one bat, we found the RNA of two different alpha CoVs (Table 2). In all other cases ($n = 30$), where we detected RNA in more than one sample of the same bat, the RNA amplified by the same PCR differed by less than 10 per cent. In 24 instances, they were 100 per cent identical; in five instances, they differed by less than 1 per cent (as a result of quasispecies replication or sequencing artifacts); and in one

instance, the difference was 5.2 per cent (potentially the result of a co-infection).

3.5 HCoV-229E-like sequences in bats

Sequences resembling HCoV-229E were found in 40 bats from the *Hipposideridae* family, primarily in the species *Hipposideros ruber*, and showed differences of up to 6 per cent among each other in the Watanabe amplicons and up to 11 per cent in the Quan amplicons. Additional sequence information of the full or partial S, E, M, and N genes was obtained, and differences in the sequences ranged up to 8 per cent for the E, 12 per cent for the M, 32 per cent for the N, and 26 per cent for the S genes. Phylogenetic
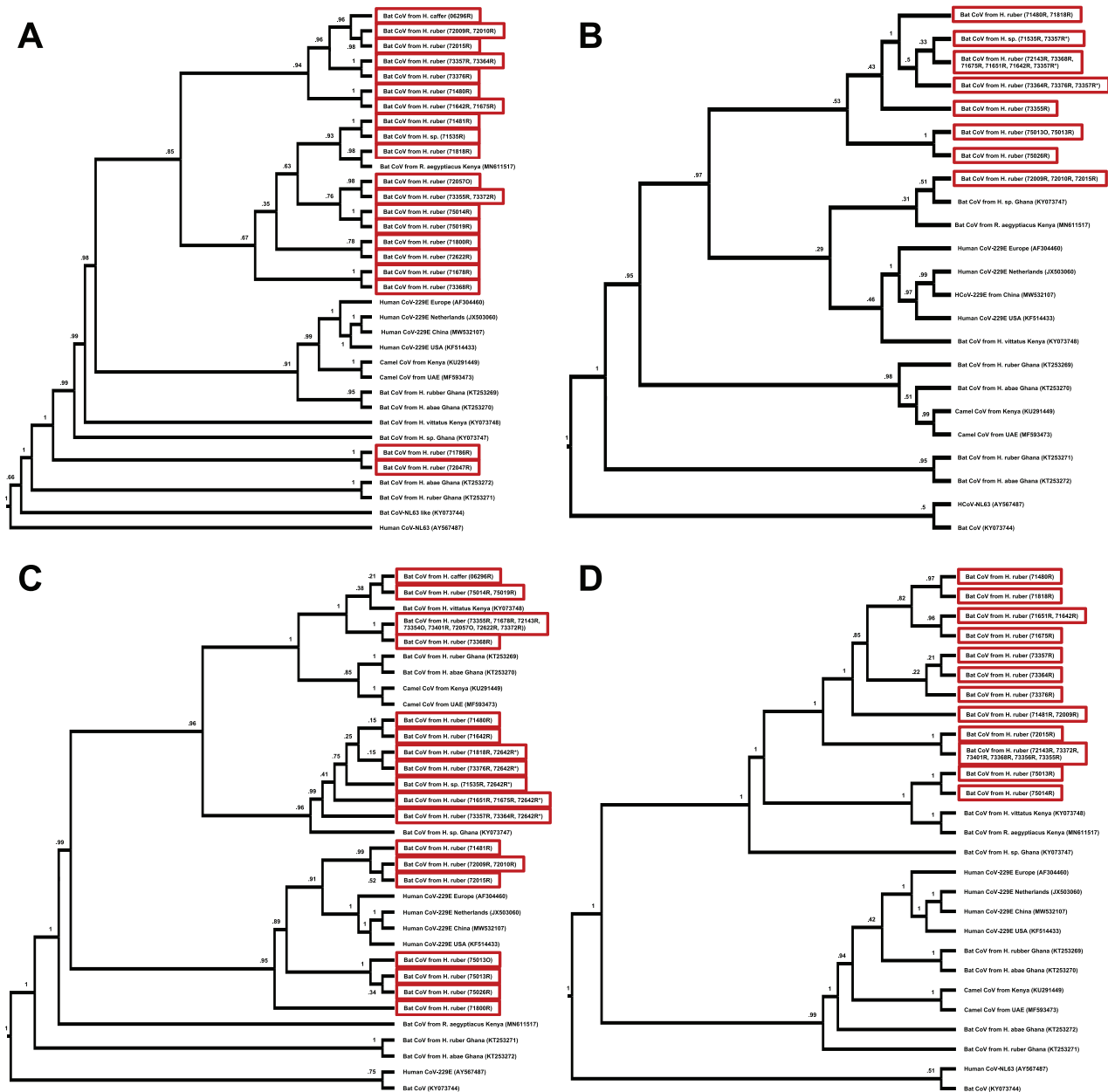
Figure 4. Phylogenetic trees of HCoV-229E-like isolates: Maximum likelihood phylogenetic tree of coronavirus sequences related to HCoC-229E based on the Spike (A), Envelope (B), Membrane (C), and Nucleoprotein (D). Red boxes indicate isolates from this study. Numbers at nodes indicate bootstrap support. Compare also Supplement 5.

clustering of sequences differed depending on the gene, with isolates BtCoV/KW2E-F56/Hip_cf._rub/GHA/2011 (KT253271) and BtCoV/AT1A-F1/Hip_aba/GHA/2010 (KT253272) being consistently the most basal isolates in the CoV-229E branch (Fig. 4, Supplement 1). The phylogenetic analyses, which involve sequence isolates from bat, camel, and human hosts, place the HCoV-229E isolates that were obtained from humans closest to isolates from camels in case of the S and N genes but closest to isolates from bats for the E, M, and RdRp genes (Fig. 4, Supplement 5).

3.6 Seasonality and other predictors in bats

Bat sample collection was focused on seasons and thus varied over the months, with a high of 432 samples collected in March and a low of 48 in August (Supplement 6). Bats sampled in the wet season accounted for 61.9 per cent of total bats compared to 38.1 per cent in the dry season. The proportion of CoV RNA-positive bats varied significantly ($P \leq 0.001$) between the wet (8.2 per cent) and dry seasons (4.5 per cent), while the proportion of events (samples collected at the same location on the same day) with at least one positive animal was very similar with 29.4 per cent during the wet season and 28.3 per cent during the dry season. The proportion of CoV RNA-positive individuals for the sampling events with >25 bats ($n = 24$) fluctuated between 0 per cent and 48 per cent and was at 21.2 per cent for the largest event. Nine events took place during the wet season, and 15 during the dry season. Among the bats sampled during this largest event were 17 different species with varying rates of CoV RNA detection, including *Chaerephon pumilus*

Table 3. PCR results of suborder, family, and species by season (bats).

Suborder, family (≥10 sampled individuals) and species (≥10 sampled individuals)	Wet season PCR positives	Dry season PCR positives	Total PCR positives
Yinpterchiroptera total[c]	7.6% (107/1412)	4.5% (36/796)	6.5% (143/2208)
Pteropodidae total[c]	6.1% (43/710)	1.6% (7/434)	4.4% (50/1144)
Eidolon helvum[b]	10.6% (12/113)	0.0% (0/154)	4.5% (12/267)
Epomophorus gambianus	37.5% (12/32)	– (0/0)	37.5% (12/32)
Epomops franqueti	1.5% (2/134)	2.8% (2/72)	1.9% (4/206)
Lissonycteris angolensis	0.0% (0/13)	0.0% (0/49)	0.0% (0/62)
Megaloglossus woermanni	3.6% (4/111)	5.5% (4/73)	4.3% (8/184)
Micropteropus pusillus	5.1% (6/117)	0.0% (0/20)	4.4% (6/137)
Myonycteris torquata	3.1% (1/32)	0.0% (0/5)	2.7% (1/37)
Rousettus aegyptiacus	4.1% (6/147)	1.9% (1/53)	3.5% (7/200)
Hipposideridae total	9.3% (63/681)	8.2% (29/354)	8.9% (92/1035)
Doryrhina cyclops	0.0% (0/34)	0.0% (0/3)	0.0% (0/37)
Macronycteris gigas[c]	8.3% (10/120)	27.9% (12/43)	13.5% (22/163)
Hipposideros ruber[b]	11.9 (50/421)	6.5% (15/232)	10.0% (65/653)
Hipposideros caffer	1.1% (1/93)	1.4% (1/72)	1.2% (2/165)
Rhinolophidae total	5.9% (1/17)	0.0% (0/8)	4.0% (1/25)
Rhinolophus landeri	0.0% (0/12)	0.0% (0/7)	0.0% (0/19)
Yangochiroptera total[c]	13.0% (24/184)	4.3% (8/188)	8.6% (32/372)
Molossidae total+	11.4% (8/70)	4.8% (7/145)	7.0% (15/215)
Chaerephon pumilus	0.0% (0/31)	– (0/0)	0.0% (0/31)
Mops condylurus[c]	22.2% (8/36)	5.0% (7/139)	8.6% (15/175)
Nycteridae total	0.0% (0/30)	0.0% (0/17)	0.0% (0/47)
Nycteris grandis	0.0% (0/8)	0.0% (0/15)	0.0% (0/23)
Nycteris hispida	0.0% (0/21)	0.0% (0/2)	0.0% (0/23)
Vespertilionidae total[a]	20.5% (16/78)	4.3% (1/23)	16.8% (17/101)
Neoromicia tenuipinnis	0.0% (0/6)	0.0% (0/7)	0.0% (0/13)
Scotophilus dinganii	16.7% (2/12)	– (0/0)	16.7% (2/12)
Scotophilus leucogaster	38.7% (12/31)	0.0% (0/2)	36.4% (12/33)
Total[c]	8.2% (131/1597)	4.5% (44/984)	6.8% (175/2581)

[a]Significant difference between calendric seasons P<0.10 (Chi-square with Yates correction).
[b]Significant difference between calendric seasons P<0.05 (Chi-square with Yates correction).
[c]Highly significant difference between calendric seasons P<0.01 (Chi-square with Yates correction).

(0 per cent, $n = 29$), *Epomophorus gambianus* (54.5 per cent, $n = 22$), *Mops condylurus* (26.7 per cent, $n = 30$), *Nycteris hispida* (0 per cent, $n = 18$), *Scotophilus dinganii* (16.7 per cent, $n = 12$), and *Scotophilus leucogaster* (38.7 per cent, $n = 31$).

Seasonal differences in the CoV RNA detection rate were associated with and differed depending on certain taxonomic species, families, and suborders (Table 3). Both *Yinpterochiroptera* and *Yangochiroptera* bats were overall more likely to be CoV RNA positive during the wet season, with the observed difference being more pronounced in the latter. Significant seasonal differences were observed in four species; high rates of CoV RNA detections were associated with the wet season and low rates with the dry season for *Eidolon helvum, Hipposideros ruber*, and *Mops condylurus*, while it was the opposite for *Macronycteris gigas* (Table 3).

4. Discussion
4.1 Unexplored diversity
While we sampled and tested roughly equal proportions of individual bats and rodents and a significant number of NHPs in this study, all but 2 of the 177 animals that turned out to be positive for CoV RNA were bats. This is not a surprise, but it rather reinforces the notion that bats are the major reservoir for alpha and beta CoVs and generally for viruses with zoonotic potential (Li et al. 2005; Woo 2009; Annan et al. 2013; O'Shea et al. 2014; Anthony et al. 2017; Geldenhuys et al. 2021). While it has been suggested that under-sampling of rodents, rather than a particularly high prevalence in bats, may be a reason for this pattern, we

did not find any evidence to support such a hypothesis in our study population, despite a high number of sampled rodents. However, considering the high diversity of rodent species, our sample is certainly not representative and biased toward those that thrive in a peridomestic environment or that are being hunted for consumption. A caveat is also that while we sampled approximately 4.1 per cent of all bat species, we only sampled 0.6 per cent of rodent species, which could have significant effects if certain species exhibit a higher prevalence of CoVs than others as it seems to be the case in bats (Geldenhuys et al. 2021). The detection of novel CoV RNA in a civet is certainly an interesting finding, as civets played a key role as intermediate hosts in the emergence of SARS-CoV-1 (Kan et al. 2005). While civets are not farmed in Cameroon, they are hunted for consumption, which implies human contact, thus posing a potential risk. The detected RNA suggests a close relationship with other carnivore CoVs and thus potentially a low risk for humans (Spillover Risk score of 54 out of 155); however, in the absence of a full genomic sequence and further characterization experiments, this remains to be determined (Grange et al. 2021).

The CoV RNA we detected belongs to 19 different genetic clusters, of which 8 might represent novel CoV species—6 of these were found in bats, 1 in a civet, and 1 in a shrew. While resource and logistical constraints prevented us from obtaining large or complete genomic sequences from the isolates, these findings indicate that the CoV diversity in African wildlife species, and particularly in bats, is still poorly understood. This is concerning since spillover events are likely to occur, given the close interactions

that arise from human housing conditions, hunting practices, eco-tourism, and other human behaviors in Cameroon and other parts of Africa. While the emergence of SARS-CoV-1 and SARS-CoV-2 have put the spotlight on Southeast Asia and China, in particular, it is important to keep in mind that the risks are present worldwide and local efforts should be enhanced across the globe to determine how to mitigate these risks (Wolfe et al. 2007; Jones et al. 2008). CoV diversity poses a significant challenge for surveillance efforts and requires further exploration, but it also provides great opportunities to learn more about the biology and history of CoVs, including that of non-SARS-CoVs, such as HCoV-229E.

4.2 Viruses closely related to HCoV-229E are highly prevalent in Hipposideros bats

We detected RNA of bat CoVs closely related to HCoV-229E in 40 bats, which all belonged to the *Hipposideridae* family (3.9 per cent, $n = 1,035$). This finding, along with the fact that previous detections of HCoV-229E-like bat viruses were also almost exclusively associated with *Hipposideros* bats, supports the hypothesis that this family of bats constitutes the original host family and reservoir of HCoV-229E (like) viruses (Corman et al. 2015). Further evidence for this is the high diversity that HCoV-229E-like viruses detected in bats exhibit compared to those found in humans or camels, which suggests a long shared evolutionary history with bats.

The observed rather low host plasticity of HCoV-229E-like viruses among bats is noteworthy since host plasticity has been proposed as a predictor for the likelihood of (successful) spillover into humans (Kreuder Johnson et al. 2015). However, in the absence of our knowledge of HCoV-229E-like viruses actually infecting camels and humans, one would potentially predict that these alpha CoVs might exhibit a lower risk for zoonotic spillover than more promiscuous bat CoVs, such as Kenya bat coronavirus BtKY56 and Eidolon bat coronavirus/Kenya/KY24 (Kumakamba et al. 2021). In that respect, there seem to be parallels between HCoV-229E and SARS-CoV-1 and SARS-CoV-2, which we believe to be primarily hosted by *Rhinolophus* bats (Li et al. 2005; Lau et al. 2005; Yip et al. 2009; Yuan et al. 2010; Hu et al. 2017; Paraskevis et al. 2020). This counterintuitive observation might be the result of a sampling bias, but considering the comparably high number of sampled animals (2,581 bats in this study alone) it is one that can hardly be ignored. Surveillance and predictions of spillover risks particularly of bat CoVs will likely play an increasing role in the aftermath of the SARS-CoV-2 pandemic but will face the challenge of limited data, despite the advances that have been made since the emergence of SARS-CoV-1.

While the zoonotic origin of globally circulating HCoV-229E is undisputed, the route by which it made its way from bats into humans is not clear. Some evidence, such as deletion patterns in the S gene and the open reading frames 4 and 8, suggests that camels may have served as an intermediate host. The hypothesis that an intermediate host was involved would be concurrent with what we know about the origins of other CoVs, including as MERS-CoV (camel) or SARS-CoV-1 (civet) (Kan et al. 2005; Corman et al. 2014, 2016; Sabir et al. 2016; Forni et al. 2017). Phylogenetic evidence, however, especially from the comparison of the more conserved genes, does not necessarily support that hypothesis. While the phylogenetic analyses of the most variable S and the N genes does place human and camel isolates into the same branch, this is not the case for the most conserved RdRp gene and the E and Mgenes (Figs 2–4, Supplement 5). This pattern would indicate that the transmission of HCoV-229E-like bat viruses into

camels (which resulted in the isolates we know to date) may have been unrelated to the spillover that eventually led to the emergence of HCoV-229E. Such a multiple spillover scenario would not be without parallel since CoVs closely related to SARS-CoV-2 were detected in pangolins but overall clearly represent a different, earlier, and unrelated transmission (Zhang et al. 2020; Lam et al. 2020). Recombination could be a factor in HCoV-229E evolution as has been proposed for this and other CoVs; however, given the partial character of the sequences we generated, we can neither confirm nor reject that this may be the case (Corman et al. 2015).

Determining the evolutionary history of HCoV-229E thus remains a challenge, not least due to limited and highly biased data available to date. Only 42 HCoV-229E isolates have been sequenced completely or near completely, with 28 derived from North America, 8 from Europe, and 7 from Southeast Asia—but not a single complete sequence from Africa. Similarly, almost all isolates from camels are derived from the Arabian peninsula and only one from Africa (Corman et al. 2016; Li et al. 2017). More research into the diversity and origins of HCoV-229E would be highly advantageous for our understanding of CoV evolution, spillover, and adaptation to humans.

4.3 Infection rates are subject to seasonality

The detection of CoVs RNA in bats has been associated with seasonal differences in the past, and we found statistically significant evidence for such a correlation in our data set as well (Anthony et al. 2017; Montecino-Latorre et al. 2020; Kumakamba et al. 2021). Much like in a recent study from the Democratic Republic of the Congo, we found that animals were more likely to be found shedding CoV RNA in the wet compared to the dry season but that this is species dependent and may be true for some but be reversed for others (Table 3) (Kumakamba et al. 2021). Aside from this overall trend, the findings regarding seasonality from the two studies do not necessarily match up; however, this may be due to different bat species sampled on the one hand and due to different ecological and climate conditions on the other hand. A potential reason for the seasonal differences may be related to the species' birthing seasons since it has been suggested that CoV transmission spikes in bat populations as juvenile bats become susceptible to infection once maternal antibody levels wane (Maganga et al. 2020; Montecino-Latorre et al. 2020). The observation that young bats in our data set were more likely to be positive for CoV RNA than adults would support this hypothesis, although our finding was not statistically significant. Given that there is not much known about the behavior and reproductive biology of the respective bat species in the specific study area(s) drawing conclusions remains challenging, although much could be learned if studies exploring the behavior and physiology of the bats of interest were to be undertaken.

Interestingly, we found female bats to be significantly more likely to be CoV RNA positive than male bats, which is the opposite of what was found in DRC and what was suspected based on behavioral differences between males and females during breeding, birthing, and breastfeeding seasons (Fayenuwo and Halstead 1974; Kumakamba et al. 2021). However, while apparently a contradiction, this might be a reflection of differences in the species composition of the data set and indicate that caution should be used when making generalizations for members of the *Chiroptera* order, even if climate and habitat are similar.

A key goal of the PREDICT work in general was to collect data about and samples from animals that are actually or potentially in close contact with humans. Part of that objective was to determine

what species can be found at the human–animal interface, and consequently the sampling targets were based on animal orders (primarily bats, rodents, and primates) rather than any particular set of species. The sample numbers for individual species are consequently not representative but rather a series of snapshots of what can be found at or near the human–animal interface in Cameroon—a matter that is true for most studies on the CoVs in African wildlife in general (Geldenhuys et al. 2021). This certainly limits the amount of meaningful statistics that can be done on many of the sampled species, and it would be desirable for future studies that want to investigate connections between ecological or behavioral trades of individual species with CoVs or other viruses to specifically define robust sampling goals for species of interest.

4.4 Closing remarks

Overall the results of this study on CoVs in African wildlife unveil or highlight several important aspects regarding the risks of future spillover and pandemics: 1) Our knowledge about the diversity of CoVs circulating in wildlife remains limited as exemplified by the fact that almost half of the CoV species we detected had not been described before. Despite having sampled 114 animal species, sample populations for most of them are too small to draw any conclusions about prevalence or risks. The role bats may play as a reservoir is certainly reinforced by the findings, but other species might simply be undersampled. The CoV RNA detection in a civet could be hinting toward a largely undetected CoV circulation in what could be an intermediate host for future spillovers. 2) The evidence for ecological factors such as seasonality driving transmission among bats is increasing. While the evidence remains circumstantial and the mechanisms elusive, it seems to become clear that further studies into this matter could enable smarter surveillance initiatives and mitigation measures, such as limiting access to caves or discouraging hunting during periods of increased virus shedding. 3) The high prevalence and diversity of HCoV-229E-like viruses in African Hipposideridae bats reinforces the notion that HCoV-229E originated in Africa. However, while more and more data supports this origin hypothesis, the question whether or not camels acted as an intermediate host during the spillover into humans remains unclear. Regardless of the intermediate host, or if there was one, HCoV-229E highlights how important it is to not focus on Southeast Asia for CoV surveillance but that CoV pandemics can start in any areas of the world.

Data availability

Additional data are available in the supplementary materials (Supplements 1–6). All viral sequences obtained are deposited in GenBank. Accession numbers for RdRp amplicons are listed in Table 2 and accession numbers for other partial genomic sequences of HCoV-229E-like viruses (MZ474745-MZ474805) are listed in Supplement 1.

Supplementary data

Supplementary data is available at *Virus Evolution* online.

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