Article

Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins

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Tommy Tsan-Yuk Lam[1,2,10], Na Jia[3,10], Ya-Wei Zhang[3,10], Marcus Ho-Hin Shum[2,10], Jia-Fu Jiang[3,10], Hua-Chen Zhu[1,2], Yi-Gang Tong[4,10], Yong-Xia Shi[5], Xue-Bing Ni[2], Yun-Shi Liao[2], Wen-Juan Li[4], Bao-Gui Jiang[3], Wei Wei[6], Ting-Ting Yuan[3], Kui Zheng[5], Xiao-Ming Cui[3], Jie Li[3], Guang-Qian Pei[3], Xin Qiang[3], William Yiu-Man Cheung[2], Lian-Feng Li[7], Fang-Fang Sun[5], Si Qin[3], Ji-Cheng Huang[5], Gabriel M. Leung[2], Edward C. Holmes[8], Yan-Ling Hu[6,9]✉, Yi Guan[1,2]✉ & Wu-Chun Cao[3]✉

The ongoing outbreak of viral pneumonia in China and across the world is associated with a new coronavirus, SARS-CoV-2[1]. This outbreak has been tentatively associated with a seafood market in Wuhan, China, where the sale of wild animals may be the source of zoonotic infection[2]. Although bats are probable reservoir hosts for SARS-CoV-2, the identity of any intermediate host that may have facilitated transfer to humans is unknown. Here we report the identification of SARS-CoV-2-related coronaviruses in Malayan pangolins (*Manis javanica*) seized in anti-smuggling operations in southern China. Metagenomic sequencing identified pangolin-associated coronaviruses that belong to two sub-lineages of SARS-CoV-2-related coronaviruses, including one that exhibits strong similarity in the receptor-binding domain to SARS-CoV-2. The discovery of multiple lineages of pangolin coronavirus and their similarity to SARS-CoV-2 suggests that pangolins should be considered as possible hosts in the emergence of new coronaviruses and should be removed from wet markets to prevent zoonotic transmission.

An outbreak of serious pneumonia disease was reported in Wuhan, China, on 30 December 2019. The causative agent was soon identified as a novel coronavirus[1], which was later named SARS-CoV-2. Case numbers grew rapidly from 27 in December 2019 to 3,090,445 globally as of 30 April 2020[3], leading to the declaration of a public health emergency, and later a pandemic, by the WHO (World Health Organization). Many of the early cases were linked to the Huanan seafood market in Wuhan city, Hubei province, from where the probable zoonotic source is speculated to originate[2]. Currently, only environmental samples taken from the market have been reported to be positive for SARS-CoV-2 by the Chinese Center for Disease Control and Prevention[4]. However, as similar wet markets were implicated in the SARS outbreak of 2002–2003[5], it seems likely that wild animals were also involved in the emergence of SARS-CoV-2. Indeed, a number of mammalian species were available for purchase in the Huanan seafood market before the outbreak[4]. Unfortunately, because the market was cleared soon after the outbreak began, determining the source virus in the animal population from the market is challenging. Although a coronavirus that is closely related to SARS-CoV-2, which was sampled from a *Rhinolophus affinis* bat in Yunnan in 2013, has now been identified[6], similar viruses have not yet been detected in other wildlife species. Here we identified SARS-CoV-2-related viruses in pangolins smuggled into southern China.

We investigated the virome composition of pangolins (mammalian order Pholidota). These animals are of growing importance and interest because they are one of the most illegally trafficked mammal species: they are used as a food source and their scales are used in traditional Chinese medicine. A number of pangolin species are now regarded as critically endangered on the International Union for Conservation of Nature Red List of Threatened Species. We received frozen tissue samples (lungs, intestine and blood) collected from 18 Malayan pangolins (*Manis javanica*) during August 2017–January 2018. These pangolins were obtained during anti-smuggling operations performed by Guangxi Customs officers. Notably, high-throughput sequencing of the RNA of these samples revealed the presence of coronaviruses in 6 out of 43 samples (2 lung samples, 2 intestinal samples, 1 lung–intestine mixed sample and 1 blood sample from 5 individual pangolins; Extended Data Table 1). With the sequence read data, and by filling gaps with amplicon sequencing, we were able to obtain six complete or near complete genome sequences—denoted GX/P1E, GX/P2V, GX/P3B, GX/P4L, GX/P5E and GX/P5L—that fall into the SARS-CoV-2 lineage (within the genus *Betacoronavirus* of the *Coronaviridae*) in a

[1]Joint Institute of Virology (Shantou University and The University of Hong Kong), Guangdong-Hongkong Joint Laboratory of Emerging Infectious Diseases, Shantou University, Shantou, P. R. China. [2]State Key Laboratory of Emerging Infectious Diseases, School of Public Health, The University of Hong Kong, Hong Kong, P. R. China. [3]State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, P. R. China. [4]Beijing Advanced Innovation Center for Soft Matter Science and Engineering (BAIC-SM), College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, P. R. China. [5]Guangzhou Customs Technology Center, Guangzhou, P. R. China. [6]Life Sciences Institute, Guangxi Medical University, Nanning, P. R. China. [7]School of Information and Management, Guangxi Medical University, Nanning, P. R. China. [8]Marie Bashir Institute for Infectious Diseases and Biosecurity, School of Life and Environmental Sciences and School of Medical Sciences, The University of Sydney, Sydney, New South Wales, Australia. [9]Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning, P. R. China. [10]These authors contributed equally: Tommy Tsan-Yuk Lam, Na Jia, Ya-Wei Zhang, Marcus Ho-Hin Shum, Jia-Fu Jiang, Yi-Gang Tong. ✉e-mail: huyanling@gxmu.edu.cn; yguan@hku.hk; caowc@bmi.ac.cn

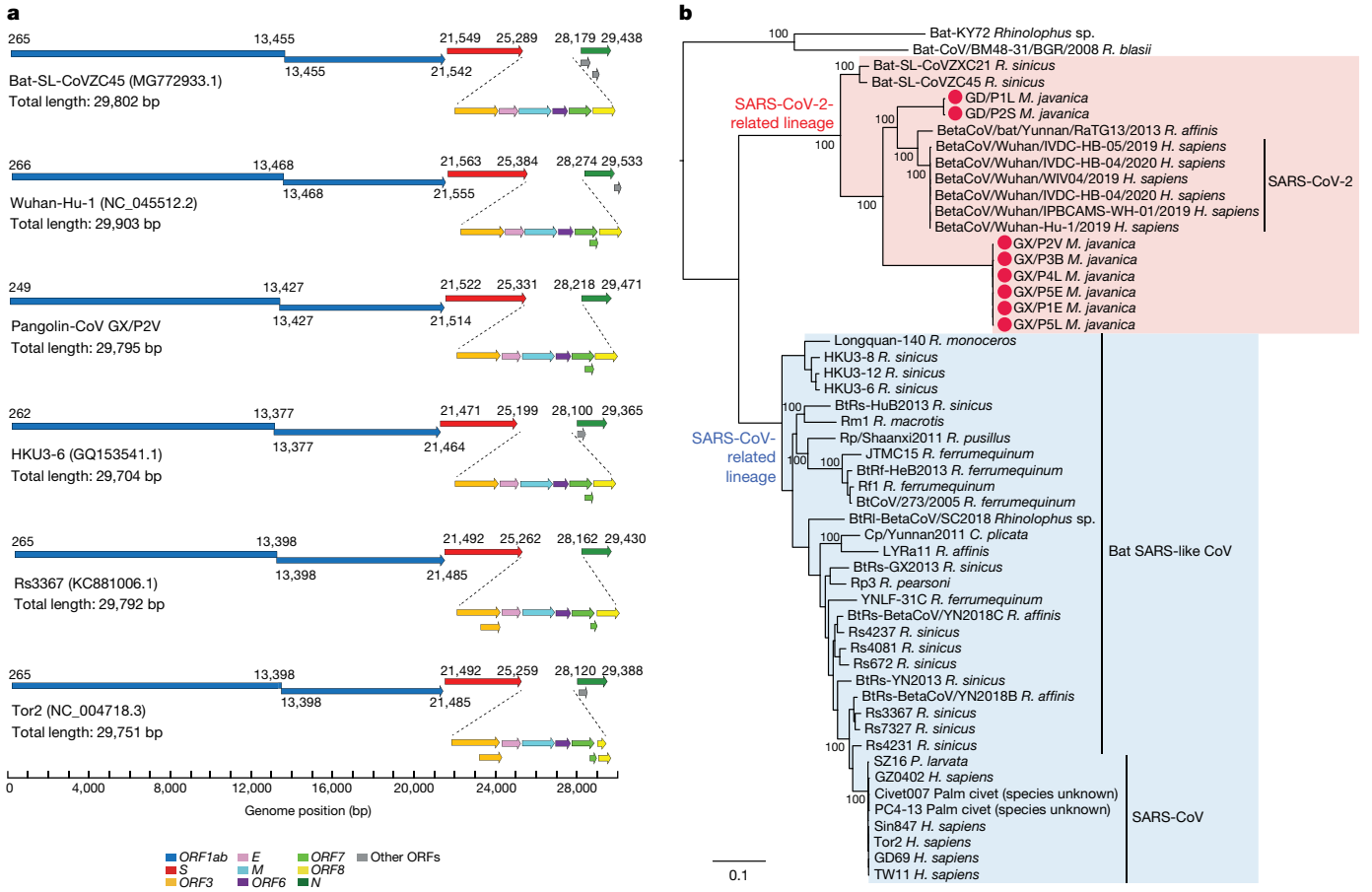

Fig. 1 | Evolutionary relationships among sequences of human SARS-CoV-2, pangolin coronaviruses and the other reference coronaviruses. a, Genome organization of coronaviruses including the pangolin coronaviruses obtained in this study, with the predicted ORFs shown in different colours (ORF1a is omitted for clarity). The pangolin coronavirus strain GX/P2V is shown with its sequence length. For comparison, the human sequences NC_045512.2 and NC_004718.3, and bat sequences MG772933.1, GQ153541.1 and KC881006.1 are included (see Extended Data Table 6 for sources). **b**, Phylogeny of the subgenus *Sarbecovirus* (genus *Betacoronavirus*; $n = 53$) estimated from the concatenated *ORF1ab*, *S*, *E*, *M* and *N* genes. Red circles indicate the pangolin coronavirus sequences generated in this study (Extended Data Table 1). GD/P1L is the consensus sequence re-assembled from previously published raw data[7]. Phylogenies were estimated using a maximum likelihood approach that used the GTRGAMMA nucleotide substitution model and 1,000 bootstrap replicates. Scientific names of the bat hosts are indicated at the end of the sequence names, and abbreviated as follows: *C. plicata*, *Chaerephon plicata*; *R. affinis*, *Rhinolophus affinis*; *R. blasii*, *Rhinolophus blasii*; *R. ferrumequinum*, *Rhinolophus ferrumequinum*; *R. monoceros*, *Rhinolophus monoceros*; *R. macrotis*, *Rhinolophus macrotis*; *R. pearsoni*, *Rhinolophus pearsoni*; *R. pusillus*, *Rhinolophus pusillus*; *R. sinicus*, *Rhinolophus sinicus*. Palm civet (*P. larvata*, *Paguma larvata*; species unspecified for Civet007 and PC4-13 sequences) and human (*H. sapiens*, *Homo sapiens*) sequences are also shown.

phylogenetic analysis (Fig. 1b). The genome sequence of the virus isolate (GX/P2V) has a very high similarity (99.83–99.92%) to the five sequences that were obtained through the metagenomic sequencing of the raw samples, and all samples have similar genomic organizations to SARS-CoV-2, with eleven predicted open-reading frames (ORFs) (Fig. 1a and Extended Data Table 2; two ORFs overlap). We were also able to successfully isolate the virus using the Vero E6 cell line (Extended Data Fig. 1). On the basis of these genome sequences, we designed primers for quantitative PCR (qPCR) detection to confirm that the raw samples were positive for coronavirus. We conducted further qPCR testing on another batch of archived pangolin samples collected between May and July 2018. Among the 19 samples (9 intestine tissues, 10 lung tissues) tested from 12 animals, 3 lung tissue samples from 3 individual pangolins were positive for coronavirus.

In addition to the animals from Guangxi, after the start of the SARS-CoV-2 outbreak researchers of the Guangzhou Customs Technology Center re-examined five archived pangolin samples (two skin swabs, two unknown tissue samples and one scale) obtained in anti-smuggling operations performed in March 2019. Following high-throughput sequencing, the scale sample was found to contain coronavirus reads, and from these data we assembled a partial genome sequence of 21,505 bp (denoted as GD/P2S), representing approximately 72% of the SARS-CoV-2 genome. Notably, this virus sequence, obtained from a pangolin scale sample, may in fact be derived from contaminants of other infected tissues. Another study of diseased pangolins in Guangdong performed in 2019 also identified viral contigs from lung samples that were similarly related to SARS-CoV-2[7]. Different assembly methods and manual curation were performed to generate a partial genome sequence that comprised 86.3% of the full-length virus genome (denoted as GD/P1L in the phylogeny shown in Fig. 1b).

These pangolin coronavirus genomes have 85.5% to 92.4% sequence similarity to SARS-CoV-2, and represent two sub-lineages of SARS-CoV-2-related viruses in the phylogenetic tree, one of which (comprising GD/P1L and GD/P2S) is very closely related to SARS-CoV-2 (Fig. 1b). It has previously been noted that members of the subgenus *Sarbecovirus* have experienced widespread recombination[8]. In support of this, a recombination analysis (Fig. 2) revealed that bat coronaviruses ZC45 and ZXC21 are probably recombinants, containing genome fragments derived from multiple SARS-CoV-related lineages (genome

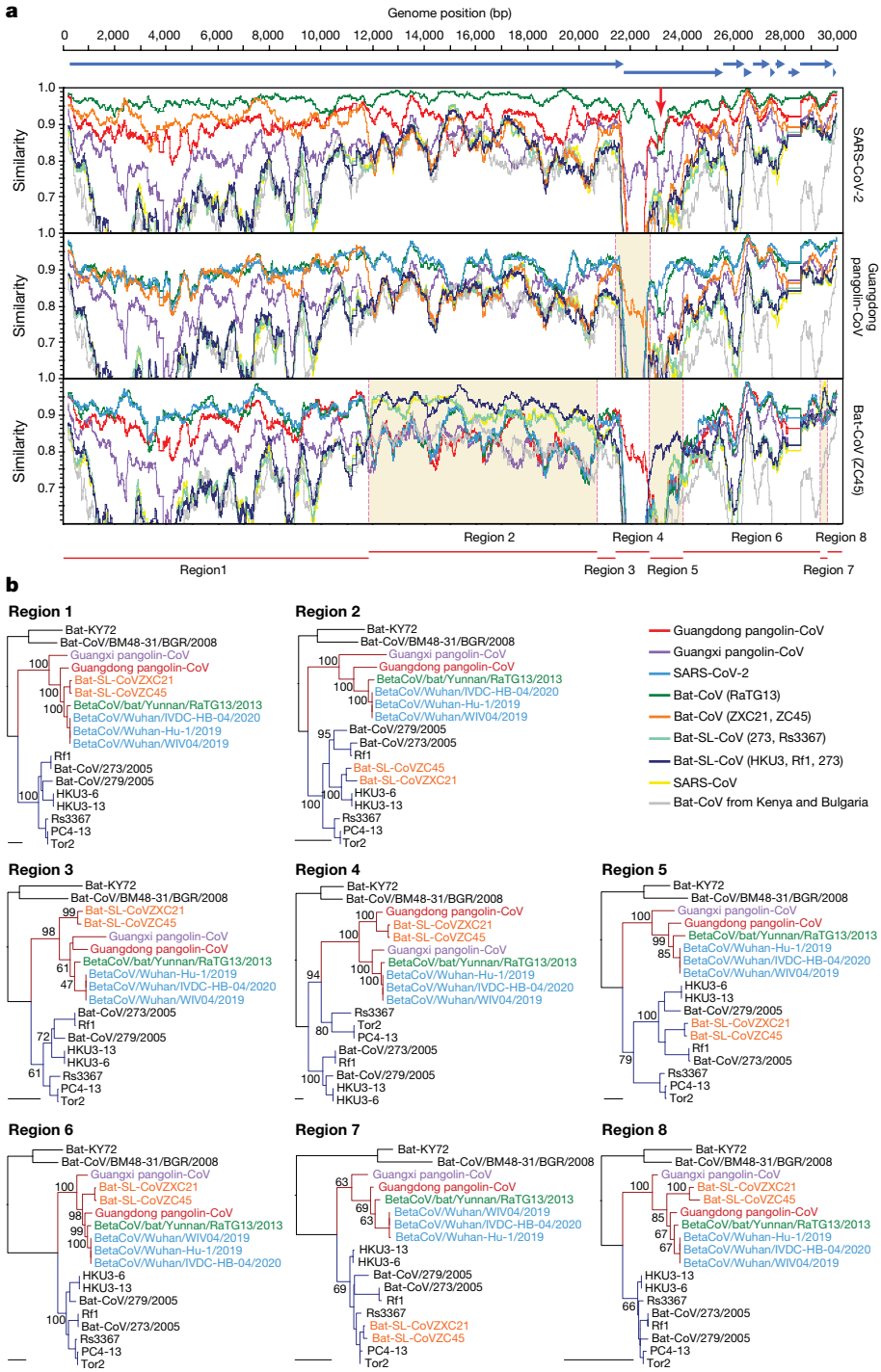

Fig. 2 | Recombination analysis. a, Sliding window analysis of changing patterns of sequence similarity between human SARS-CoV-2, pangolin and bat coronaviruses. The potential recombination breakpoints are shown in pink dash lines, and regions separated by the breakpoints are alternatively shaded in yellow. These potential breakpoints subdivide the genomes into eight regions (regions with fewer than 200 bp were omitted), indicated by the red bars at the bottom of the analysis boxes. The names of the query sequences are shown vertically to the right of the analysis boxes. The similarities to different reference sequences are indicated by different colours. Guangdong pangolin-CoV GD/P1L and pangolin-CoV GD/P2S were merged for this analysis. The blue arrows at the top indicate the position of the ORFs in the alignment. **b**, Phylogenetic trees of different genomic regions. SARS-CoV- and SARS-CoV-2-related lineages are shown in blue and red tree branches, respectively. Branch supports obtained from 1,000 bootstrap replicates are shown. Branch scale bars are shown as 0.1 substitutions per site.

regions 2, 5 and 7) as well as SARS-CoV-2-related lineages, including segments from pangolin coronaviruses (regions 1, 3, 4, 6 and 8).

More notable, however, was the observation of putative recombination signals between the pangolin coronaviruses, bat coronavirus RaTG13 and human SARS-CoV-2 (Fig. 2). In particular, SARS-CoV-2 exhibits very high sequence similarity to the Guangdong pangolin coronaviruses in the receptor-binding domain (RBD) (97.4% amino acid similarity, indicated by red arrow in Fig. 2a; the alignment is shown in Fig. 3a), even though it is most closely related to bat coronavirus RaTG13 in the remainder of the viral genome. Indeed, the Guangdong pangolin coronaviruses and SARS-CoV-2 possess identical amino acids at the five critical residues of the RBD, whereas RaTG13 only shares one amino acid with SARS-CoV-2 (residue 442, according to numbering of the human SARS-CoV[9]) and these latter two viruses have only 89.2% amino acid similarity in the RBD. Notably, a phylogenetic analysis of synonymous sites only from the RBD revealed that the topological position of the Guangdong pangolin is consistent with that of the remainder of the viral genome, rather than being the closest relative of SARS-CoV-2 (Fig. 3b). Therefore, it is possible that the amino acid similarity between the RBD of the Guangdong pangolin coronaviruses and SARS-CoV-2 is due to selectively mediated convergent evolution rather than recombination, although it is difficult to differentiate between these scenarios on the basis of the current data. This observation is consistent with the fact that the sequence similarity of ACE2 is higher between humans and pangolins (84.8%) than between humans and bats (80.8–81.4% for *Rhinolophus* sp.) (Extended Data Table 3). The occurrence of recombination and/or convergent evolution further highlights the role that intermediate animal hosts have in the emergence of viruses that can

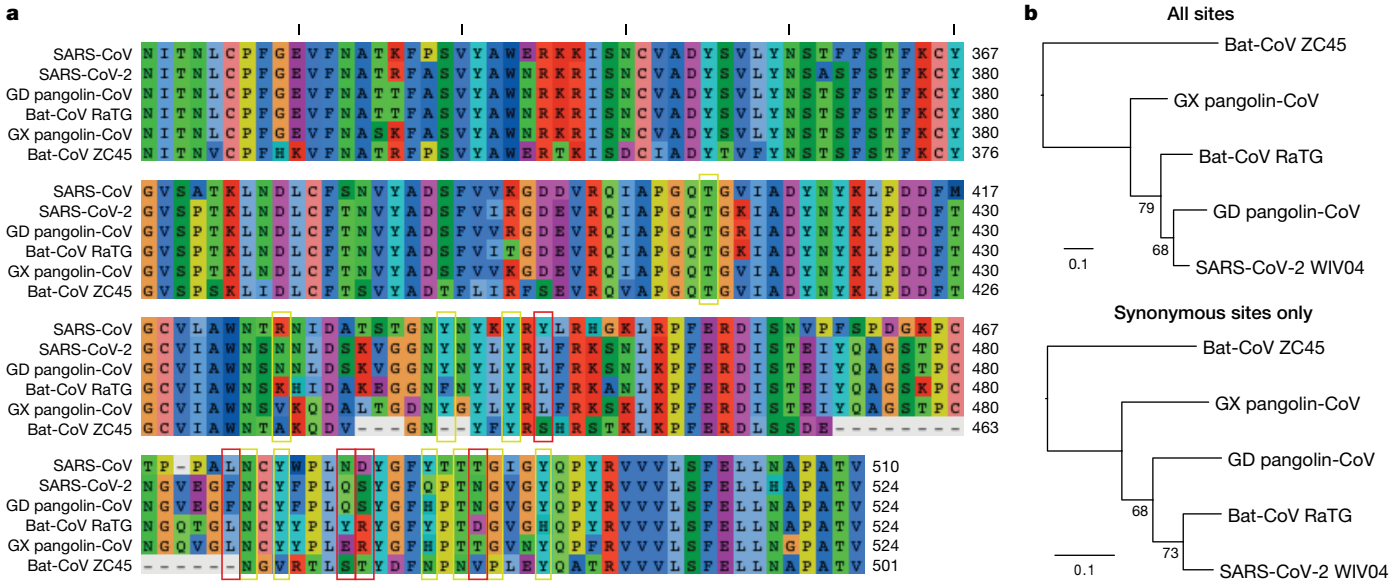

Fig. 3 | Analysis of the RBD sequence. a, Sequence alignment showing the RBD in human, pangolin and bat coronaviruses. The five critical residues for binding between SARS-CoV RBD and human ACE2 protein are indicated in red boxes, and ACE2-contacting residues are indicated by yellow boxes as previously described[9]. In the Guangdong pangolin-CoV sequence, the codon positions encoding the amino acids Pro337, Asn420, Pro499 and Asn519 have ambiguous nucleotide compositions, resulting in possible alternative amino acids at these sites (threonine, glycine, threonine and lysine, respectively). Sequence gaps are indicated with dashes. The short black lines at the top indicate the positions of every 10 residues. GD, Guangdong; GX, Guangxi. **b**, Phylogenetic trees of the SARS-CoV-2-related lineage estimated from the entire RBD region (top) and synonymous sites only (bottom). Branch supports obtained from 1,000 bootstrap replicates are shown. Branch scale bars are shown as 0.1 substitutions per site.

infect humans. However, all of the pangolin coronaviruses identified to date lack the insertion of a polybasic (furin-like) S1/S2 cleavage site in the spike protein that distinguishes human SARS-CoV-2 from related betacoronaviruses (including RaTG13)[10] and that may have helped to facilitate the emergence and rapid spread of SARS-CoV-2 through human populations.

To our knowledge, pangolins are the only mammals in addition to bats that have been documented to be infected by a SARS-CoV-2-related coronavirus. It is notable that two related lineages of coronaviruses are found in pangolins that were independently sampled in different Chinese provinces and that both are also related to SARS-CoV-2. This suggests that these animals may be important hosts for these viruses, which is surprising as pangolins are solitary animals that have relatively small population sizes, reflecting their endangered status[11]. Indeed, on the basis of the current data it cannot be excluded that pangolins acquired their SARS-CoV-2-related viruses independently from bats or another animal host. Therefore, their role in the emergence of human SARS-CoV-2 remains to be confirmed. In this context, it is noteworthy that both lineages of pangolin coronaviruses were obtained from trafficked Malayan pangolins, which originated from Southeast Asia, and that there is a marked lack of knowledge of the viral diversity maintained by this species in regions in which it is indigenous. Furthermore, the extent of virus transmission in pangolin populations should be investigated further. However, the repeated occurrence of infections with SARS-CoV-2-related coronaviruses in Guangxi and Guangdong provinces suggests that this animal may have an important role in the community ecology of coronaviruses.

Coronaviruses, including those related to SARS-CoV-2, are present in many wild mammals in Asia[5–7,12]. Although the epidemiology, pathogenicity, interspecies infectivity and transmissibility of coronaviruses in pangolins remains to be studied, the data presented here strongly suggests that handling these animals requires considerable caution and their sale in wet markets should be strictly prohibited. Further surveillance of pangolins in their natural environment in China and Southeast Asia are necessary to understand their role in the emergence of coronaviruses and the risk of future zoonotic transmissions.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2169-0.

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Methods

Data reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Ethics statement

The animals studied here were rescued and treated by the Guangxi Zhuang Autonomous Region Terrestrial Wildlife Medical-aid and Monitoring Epidemic Diseases Research Center under the ethics approval (wild animal treatment regulation No. [2011] 85). The samples were collected following the procedure guideline (Pangolins Rescue Procedure, November, 2016).

Sample collection, viral detection and sequencing of pangolins in Guangxi

We received frozen tissue samples of 18 pangolins (*M. javanica*) from Guangxi Medical University, China, that were collected between August 2017 – January 2018. These pangolins were seized by the Guangxi Customs during their routine anti-smuggling operations. All animal individuals comprised samples from multiple organs including lungs, intestine and blood, with the exception of six individuals for which only lung tissues were available, five with mixed intestine and lung tissues only, one with intestine tissues only, and one comprising two blood samples. Using the intestine–lung mixed sample we were able to isolate a novel *Betacoronavirus* using the Vero-E6 cell line (from ATCC; Extended Data Fig. 1). The cell line was subjected to species identification and authentication by microscopic morphologic evaluation and growth curve analysis, and was tested free of mycoplasma contamination. The cell line was not on the list of common misidentified cell lines by ICLAC. A High Pure Viral RNA Kit (Roche) was used for RNA extraction on all 43 samples. For RNA sequencing (GX/P2V and GX/P3B), a sequencing library was constructed using an Ion Total RNA-Seq Kit v2 (Thermo Fisher Scientific), and the library was subsequently sequenced using an Ion Torrent S5 sequencer (Thermo Fisher Scientific). For other samples, reverse transcription was performed using an SuperScript III First-Strand Synthesis System for RT–PCR (Thermo Fisher Scientific). DNA libraries were constructed using the NEBNext Ultra II DNA Library Prep Kit and sequenced on a MiSeq sequencer. The NGS (next-generation sequencing) QC Toolkit V2.3.3 was used to remove low-quality and short reads. Both BLASTn and BLASTx were used to search against a local virus database, using the data available at NCBI/GenBank. Genome sequences were assembled using the CLC Genomic Workbench v.9.0. To fill gaps in high throughput sequencing and obtain the whole viral genome sequence, amplicon primers based on the bat SARS-like coronavirus ZC45 (GenBank accession number MG772933) sequence and the coronavirus contigs obtained in the initial sequencing were designed for further amplicon-based sequencing.

A total of six samples (including the virus isolate) contained reads that matched members of the genus *Betacoronavirus* (Extended Data Table 1). We obtained near complete viral genomes from these samples (98%, compared to SARS-CoV-2), which were designated GX/P1E, GX/P2V, GX/P3B, GX/P4L, GX/P5E and GX/P5L. Their average sequencing coverage ranged from approximately 8.4X to 8,478X (Extended Data Fig. 2a-f). On the basis of these genome sequences, we designed primers for qPCR to confirm the positivity of the original tissue samples (Extended Data Table 4). This revealed an original lung tissue sample that was also qPCR positive, in addition to the six original samples with coronavirus reads. We further tested an additional 19 samples (nine intestine tissues and ten lung tissues), from 12 smuggled pangolins sampled between May–July 2018 by the group from Guangxi Medical University. The genome sequences of GX/P1E, GX/P2V, GX/P3B, GX/P4L, GX/P5E and GX/P5L have been submitted to GISAID database and assigned accession numbers EPI_ISL_410538 - EPI_ISL_410543.

Sample collection, viral detection and sequencing of pangolins in Guangdong

After the start of the SARS-CoV-2 outbreak, the Guangzhou Customs Technology Center re-examined their five archived pangolin samples (two skin swabs, two unknown tissue and one scale) obtained in anti-smuggling operations undertaken in March 2019. RNA was extracted from all five samples (Qiagen), and was subjected to high-throughput RNA sequencing on the Illumina HiSeq platform by Vision medicals. The scale sample was found to contain coronavirus reads using a BLAST-based approach. These reads were quality assessed, cleaned and assembled into contigs by both de novo (MEGAHIT v1.1.3[13]) and using reference (BWA v0.7.13[14]) assembly methods, using BetaCoV/Wuhan/WIV04/2019 as a reference. The contigs were combined, and approximately 72% of the coronavirus genome (21,505 bp) was obtained. This sequence has about 6.6× sequencing coverage (Extended Data Fig. 2g) and denoted pangolin-CoV GD/P2S. This sequence has been deposited on GISAID with accession number EPI_ISL_410544.

A recently published meta-transcriptomic study of pangolins[7] deposited 21 RNA-seq raw files on the SRA database (https://www.ncbi.nlm.nih.gov/sra). We screened these raw read files using BLAST methods and found that five (SRR10168374, SRR10168376, SRR10168377, SRR10168378 and SRR10168392) contained reads that mapped to SARS-CoV-2. These reads were subjected to quality assessment, cleaning and then de novo assembly using MEGAHIT[13] and reference assembly using BWA[14]. These reads were then merged and curated in a pileup alignment file to obtain the consensus sequences. This combined consensus sequence is 25,753 bp in length (about 86.3% of BetaCoV/Wuhan/WIV04/2019; about 6.9× coverage) and denoted pangolin-CoV GD/P1L (available in the Supplementary Information Dataset). Notably, it has 66.8% overlap and a sequence identity of 99.79% with the GD/P2S sequence. As the genetic distance between these viruses is very low, for the recombination analysis we merged the GD/P1L and GD/P2S sequences into a single consensus sequence to minimize gap regions within any sequences.

The viral genome organizations of the Guangxi and Guangdong pangolin coronaviruses were similar to SARS-CoV-2. They possessed nine non-overlapping open reading frames (ORFs) plus two overlapping ORFs, and shared the same gene order of ORF1ab replicase, envelope glycoprotein spike (S), envelope (E), membrane (M), nucleocapsid (N), plus other predicted ORFs. A detailed comparison of the ORF length and similarity with SARS-CoV-2 and bat coronavirus RaTG13 is provided in Extended Data Table 2.

Sequence, phylogenetic and recombination analyses

The human SARS-CoV-2 and bat RaTG13 coronavirus genome sequences were downloaded from Virological.org (http://virological.org) and the GISAID (https://www.gisaid.org) databases in January 2020, with the data kindly shared by the submitters (Extended Data Table 5). Other coronaviruses (subgenus *Sarbecovirus*) were downloaded from GenBank (Extended Data Table 6) and compared to those obtained here. We constructed a multiple sequence alignment of their complete genomes and individual genes using MAFFT v.7.273[15]. Maximum likelihood phylogenies were estimated using RAxML v.8.2.12[16] from 100 inferences, using the GTRGAMMA model of nucleotide substitution with 1,000 bootstrap replicates. To investigate potential recombination events, we used SimPlot v.3.5.1[17] to conduct a window sliding analysis to determine the changing patterns of sequence similarity and phylogenetic clustering between the query and the reference sequences. A full plot for the recombination analysis is provided in Extended Data Fig. 3. We also examined phylogenetic clusters performed directly from the multiple sequence alignment. Maximum likelihood trees were estimated from each window extraction (that is, genome regions 1 to 8) using RAxML as described above.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Data that support the findings of this study have been deposited in the GISAID database (https://www.gisaid.org) with accession numbers EPI_ISL_410538–EPI_ISL_410544 and the SRA database under BioProject accession number PRJNA606875. The data are also available as Supplementary Information.

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Author contributions W.-C.C., N.J., J.-C.H., Y.G. and Y.-L.H. designed and supervised the research. J.-F.J., B.-G.J., W.W., T.-T.Y., K.Z., L.-F.L. and X.-M.C. collected samples. Y.-W.Z., Y.-X.S., W.-J.L., W.W., T.-T.Y., J.L. and L.-F.L. prepared materials for sequencing. Y.-W.Z., Y.-X.S., G.-Q.P., X.Q., F.-F.S. and S.Q. performed genome sequencing. Y.-W.Z., M.H.-H.S., X.-B.N. and T.T.-Y.L. performed genome assembly and annotation. Y.-G.T., T.T.-Y.L., M.H.-H.S., Y.-W.Z., X.-B.N., E.C.H., Y.-S.L. and N.J. performed the genome analysis and interpretation. T.T.-Y.L., N.J., E.C.H. and W.-C.C. wrote the paper. All authors took part in data interpretation and edited the paper.

Competing interests The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Y.-L.H., Y.G. or W.-C.C.

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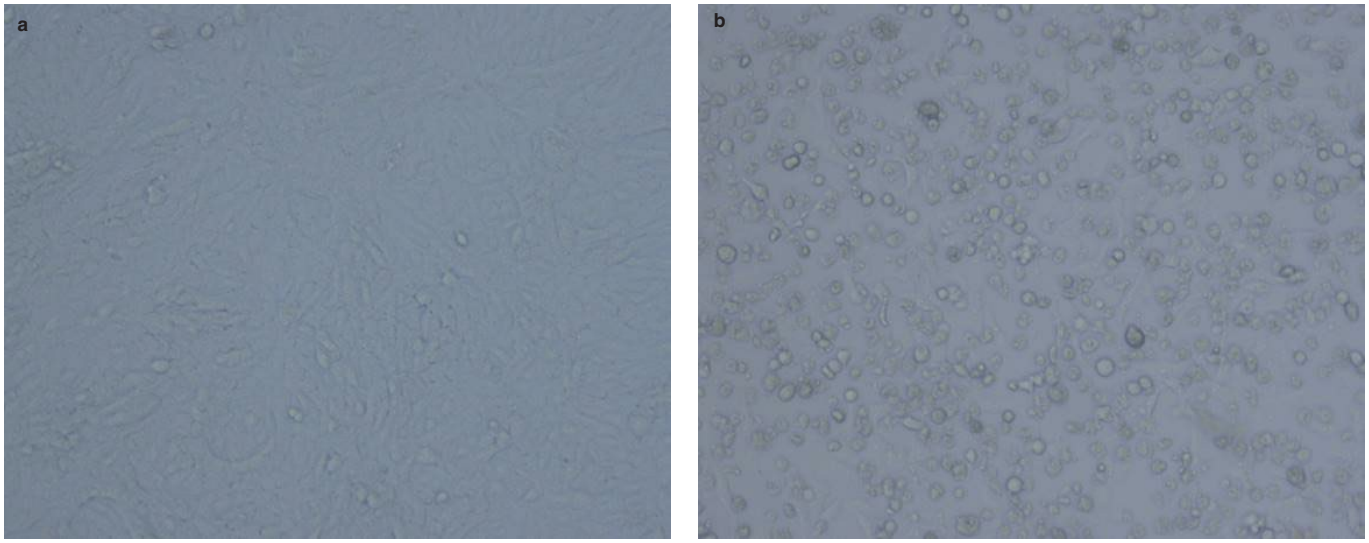
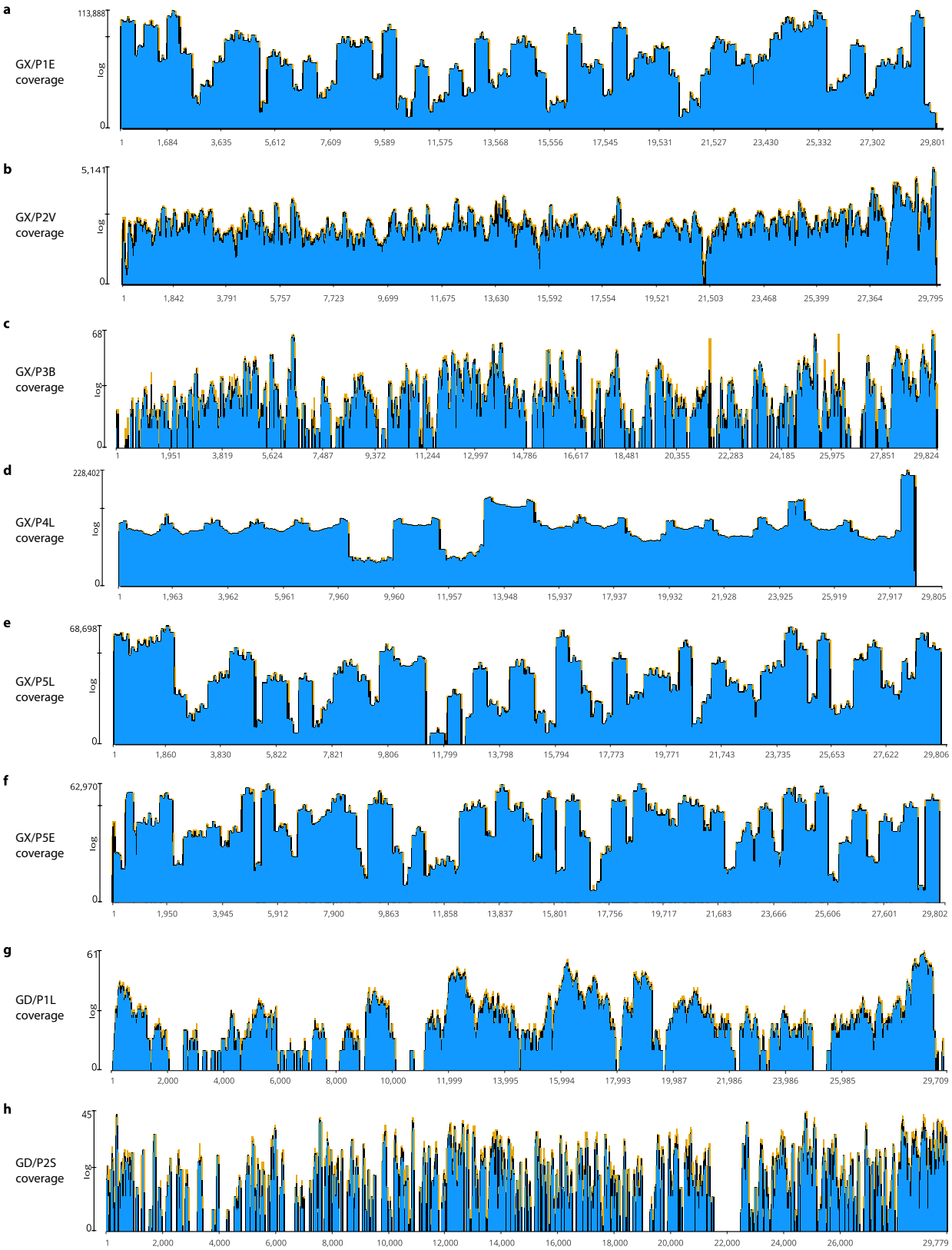
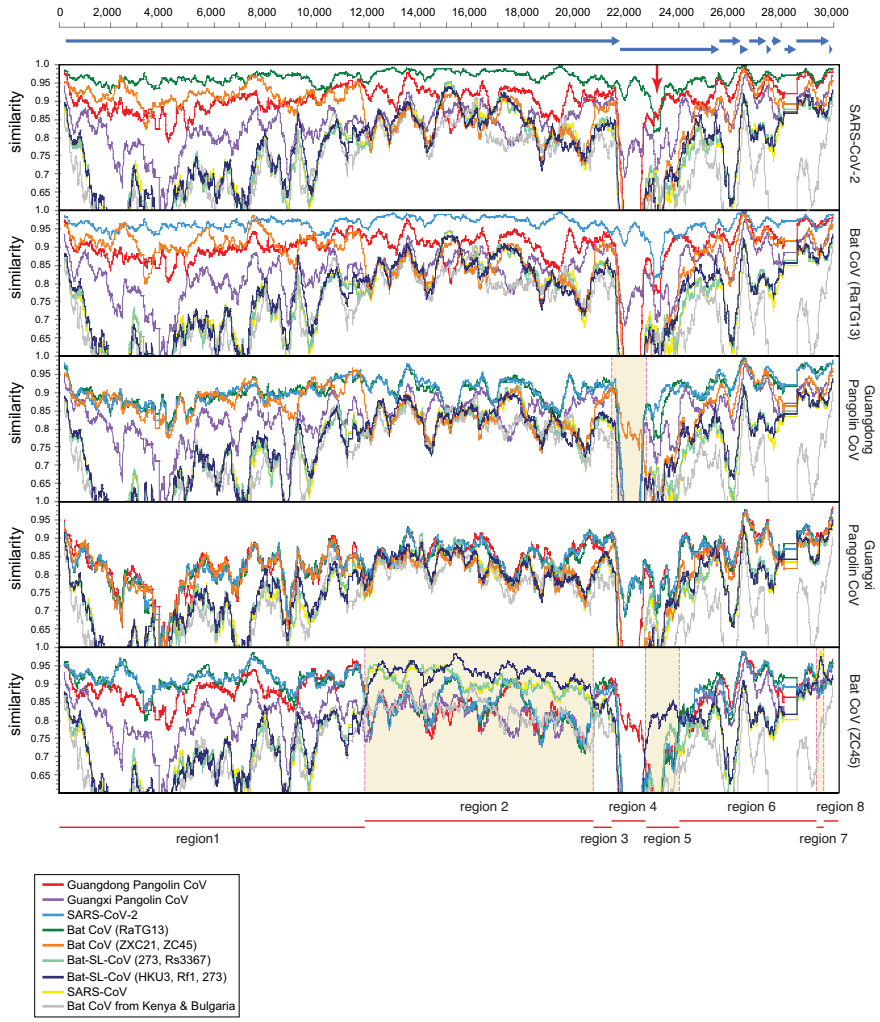


Extended Data Fig. 1 | Microscopy image of the cytopathic effect of the virus in Vero E6 cells. a, Negative control. Uninfected cells of the Vero E6 cell line. **b**, Cytopathic effect seen in viral culture (five days after inoculation). The experiment was performed twice independently in two laboratories and produced similar results.



Extended Data Fig. 2 | Read coverage depth of each pangolin coronavirus analysed in this study. a, GX/P1E. **b**, GX/P2V. **c**, GX/P3B. **d**, GX/P4L. **e**, GX/P5L. **f**, GX/P5E. **g**, GD/P1L. **h**, GD/P2S.



Extended Data Fig. 3 | Recombination analysis of all members of the SARS-CoV-2-related lineages. Sliding window analysis of changes in the patterns of sequence similarity between human SARS-CoV-2, and pangolin and bat coronaviruses as described further in Fig. 2a. Sequence similarity patterns of Bat-CoV (RaTG13) and Guangxi pangolin-CoV are shown in this figure but not in Fig. 2a.

Extended Data Table 1 | High-throughput sequencing results of the pangolin samples with coronavirus reads

Source location	Sample type	Sample number	Accession IDs of consensus sequence / read data
Guangxi	Intestine	GX/P1E	EPI_ISL_410539 / SAMN14115945
Guangxi	Virus isolate from intestine-lung mixed samples	GX/P2V	EPI_ISL_410542 / SAMN14115940
Guangxi	Blood	GX/P3B	EPI_ISL_410543 / SAMN14115941
Guangxi	Lung	GX/P4L	EPI_ISL_410538 / SAMN14115942
Guangxi	Intestine	GX/P5E	EPI_ISL_410541 / SAMN14115943
Guangxi	Lung	GX/P5L	EPI_ISL_410540 / SAMN14115944
Guangdong	Scale	GD/P2S	EPI_ISL_410544 / SAMN14116618

Sequencing reads have been deposited in the SRA database under BioProject accession number PRJNA606875.

Extended Data Table 2 | Genomic comparison of SARS-CoV-2 with bat-CoV RaTG13, Guangdong pangolin-CoV and Guangxi pangolin-CoV

	Bat-Cov RaTG13[#]			Guangdong pangolin CoV [#]			Guangxi pangolin CoV [#]		
	Length bat/ SARS-CoV-2 (bp)	nt Identity %	aa Identity %	Length GD/ SARS-CoV-2 (bp)	nt Identity %	aa Identity %	Length GX/ SARS-CoV-2 (bp)	nt Identity %	aa Identity %
ORF1ab	21287/21290	96.5	98.6	20076*/21290	90.7	97.1	21266/21290	84.9	92.5
S	3810/3822	93.1	97.7	3548*/3822	84.9	90.7	3804/3822	83.6	92.6
ORF3a	828/828	96.3	97.8	828/828	93.6	97.4	828/828	87.0	89.3
E	228/228	99.6	100	228/228	99.1	100	228/228	97.4	100
M	666/669	95.9	100	669/669	93.4	98.6	669/669	91.3	98.2
ORF6	186/186	98.4	100	186/186	95.7	96.6	186/186	90.9	95.0
ORF7a	366/366	95.6	97.5	366/366	93.4	97.5	366/366	86.6	87.7
ORF8	366/366	97.0	94.9	366/366	92.3	94.9	366/366	80.6	86.8
N	1260/1260	96.9	99.0	1260/1260	96.2	97.8	1254/1260	91.4	94.3

*Partial sequence.

[#]Wuhan-Hu-1 SARS-CoV-2 (NC_045512.2) was used for comparison with bat-CoV RaTG13 (EPI_ISL_402131), Guangdong pangolin-CoV (merge of GD/P1L and GD/P2S) and Guangxi pangolin-CoV (GX/P5L).

Extended Data Table 3 | Sequence similarity of amino acid sequences of ACE2 between humans, pangolins and bats

	Homo sapiens	*Manis javanica*	*Rhinolophus sinicus*	*Rhinolophus pearsonii*	*Rhinolophus ferrumequinum*
Homo sapiens	100%				
Manis javanica	84.85%	100%			
Rhinolophus sinicus	80.75%	82.86%	100%		
Rhinolophus pearsonii	81.37%	82.98%	94.41%	100%	
Rhinolophus ferrumequinum	81.24%	82.98%	93.04%	92.42%	100%
Rhinolophus macrotis	80.87%	83.73%	95.78%	94.91%	92.55%

Extended Data Table 4 | Primers used for qPCR detection of pangolin-associated coronaviruses

pCov-Forward	AGGTGACGAGGTTAGACAAATAG
pCov-Reverse	CCAAGCAATAACACAACCAGTAA
pCov-Probe	ACCCGGACAAACTGGTGTTATTGCT

Extended Data Table 5 | Previously obtained SARS-CoV-2 genome sequences

Accession ID	Virus name	Location	Collection date	Originating lab	Submitting lab	Authors
Virologal.org sequence (NC_045512.2)	BetaCoV/Wuhan-Hu-1/2019	China / Wuhan	2019-12	National Institute for Communicable Disease Control and Prevention (ICDC) Chinese Center for Disease Control and Prevention (China CDC)	National Institute for Communicable Disease Control and Prevention (ICDC) Chinese Center for Disease Control and Prevention (China CDC)	Zhang,Y.-Z., Wu,F., Chen,Y.-M., Pei,Y.-Y., Xu,L., Wang,W., Zhao,S., Yu,B., Hu,Y., Tao,Z.-W., Song,Z.-G., Tian,J.-H., Zhang,Y.-L., Liu,Y., Zheng,J.-J., Dai,F.-H., Wang,Q.-M., She,J.-L. and Zhu,T.-Y.
EPI_ISL_ 402131	BetaCoV/bat/ Yunnan/ RaTG13/2013	China / Yunnan Province / Pu'er City	2013-07-24	Wuhan Institute of Virology, Chinese Academy of Sciences	Wuhan Institute of Virology, Chinese Academy of Sciences	Yan Zhu, Ping Yu, Bei Li, Ben Hu, Hao-Rui Si, Xing-Lou Yang, Peng Zhou, Zheng-Li Shi
EPI_ISL_ 402121	BetaCoV/Wuhan/ IVDC-HB-05/2019	China / Hubei Province / Wuhan City	2019-12-30	National Institute for Viral Disease Control and Prevention, China CDC	National Institute for Viral Disease Control and Prevention, China CDC	Wenjie Tan，Xuejun Ma，Xiang Zhao，Wenling Wang，Yongzhong Jiang，Roujian Lu，Ji Wang，Peihua Niu, Weimin Zhou, Faxian Zhan，Weifeng Shi，Baoying Huang，Jun Liu，Li Zhao，Yao Meng，Fei Ye，Na Zhu, Xiaozhou He，Peipei Liu, Yang Li，Jing Chen，Wenbo Xu，George F. Gao，Guizhen Wu
EPI_ISL_ 402120	BetaCoV/Wuhan/ IVDC-HB-04/2020	China / Hubei Province / Wuhan City	2020-01-01	National Institute for Viral Disease Control and Prevention, China CDC	National Institute for Viral Disease Control and Prevention, China CDC	Wenjie Tan，Xiang Zhao，Wenling Wang，Xuejun Ma，Yongzhong Jiang，Roujian Lu，Ji Wang，Weimin Zhou，Peihua Niu，Peipei Liu，Faxian Zhan，Weifeng Shi，Baoying Huang，Jun Liu，Li Zhao，Yao Meng，Xiaozhou He，Fei Ye，Na Zhu，Yang Li，Jing Chen，Wenbo Xu，George F. Gao，Guizhen Wu
EPI_ISL_ 402124	BetaCoV/Wuhan/ WIV04/2019	China / Hubei Province / Wuhan City	2019-12-30	Wuhan Jinyintan Hospital	Wuhan Institute of Virology, Chinese Academy of Sciences	Peng Zhou, Xing-Lou Yang, Ding-Yu Zhang, Lei Zhang, Yan Zhu, Hao-Rui Si, Zhengli Shi
EPI_ISL_ 402123	BetaCoV/Wuhan /IPBCAMS-WH-01/2019	China / Hubei Province / Wuhan City	2019-12-24	Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College	Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College	Lili Ren, Jianwei Wang, Qi Jin, Zichun Xiang, Zhiqiang Wu, Chao Wu, Yiwei Liu

SARS-CoV-2 genome sequences are available at virological.org and in the GISAID (https://www.gisaid.org) databases.

Extended Data Table 6 | GenBank accession numbers of coronavirus sequences used in this study

Accession ID	Strain name	Host	Publication
NC_004718.3	Tor2	*Homo sapiens*	He *et al.* Biochem Biophys Res Commun. 316(2):476-83 (2004) [18] Snijder *et al.* J. Mol. Biol. 331 (5), 991-1004 (2003) [19] Marra *et al.* Science 300 (5624), 1399-1404 (2003) [20]
AY313906.1	GD69	*Homo sapiens*	Song *et al.* Proc. Natl. Acad. Sci. U. S. A. 102(7):2430-5 (2005) [21]
MK211377.1	BtRs-BetaCoV/YN2018C	*Rhinolophus affinis*	Han *et al.* Front Microbiol. 10:1900 (2019)[22]
MK211376.1	BtRs-BetaCoV/YN2018B	*Rhinolophus affinis*	Han *et al.* Front Microbiol. 10:1900 (2019) [22]
MK211374.1	BtRl-BetaCoV/SC2018	*Rhinolophus sp.*	Han *et al.* Front Microbiol. 10:1900 (2019) [22]
KY352407.1	BtKY72	*Rhinolophus sp.*	Tao *et al.* Microbiol Resour Announc 8 (28), e00548-19 (2019) [23]
MG772934.1	bat-SL-CoVZXC21	*Rhinolophus sinicus*	Hu et al. Emerg Microbes Infect. 12;7(1):154 (2018) [24]
MG772933.1	bat-SL-CoVZC45	*Rhinolophus sinicus*	Hu et al. Emerg Microbes Infect. 12;7(1):154 (2018) [24]
KY417151.1	Rs7327	*Rhinolophus sinicus*	Hu *et al.* PLoS Pathog. 13 (11), e1006698 (2017) [25]
KY417147.1	Rs4237	*Rhinolophus sinicus*	Hu *et al.* PLoS Pathog. 13 (11), e1006698 (2017) [25]
KY417146.1	Rs4231	*Rhinolophus sinicus*	Hu *et al.* PLoS Pathog. 13 (11), e1006698 (2017) [25]
KY417143.1	Rs4081	*Rhinolophus sinicus*	Hu *et al.* PLoS Pathog. 13 (11), e1006698 (2017) [25]
KJ473816.1	BtRs-YN2013	*Rhinolophus sinicus*	Wu *et al.* J. Infect. Dis. 213 (4), 579-583 (2016) [26] Wu *et al.* ISME J 10 (3), 609-620 (2016) [27]
KJ473815.1	BtRs-GX2013	*Rhinolophus sinicus*	Wu *et al.* J. Infect. Dis. 213 (4), 579-583 (2016) [26] Wu *et al.* ISME J 10 (3), 609-620 (2016) [27]
KJ473814.1	BtRs-HuB2013	*Rhinolophus sinicus*	Wu *et al.* J. Infect. Dis. 213 (4), 579-583 (2016) [26] Wu *et al.* ISME J 10 (3), 609-620 (2016) [27]
KJ473812.1	BtRf-HeB2013	*Rhinolophus ferrumequinum*	Wu *et al.* J. Infect. Dis. 213 (4), 579-583 (2016) [26] Wu *et al.* ISME J 10 (3), 609-620 (2016) [27]
JX993988.1	Cp/Yunnan2011	*Chaerephon plicata*	Yang *et al.* Emerging Infect. Dis. 19 (6) (2013) [28] Wu *et al.* J. Infect. Dis. 213 (4), 579-583 (2016) [26] Wu *et al.* ISME J 10 (3), 609-620 (2016) [27]
JX993987.1	Rp/Shaanxi2011	*Rhinolophus pusillus*	Yang *et al.* Emerging Infect. Dis. 19 (6) (2013) [28] Wu *et al.* J. Infect. Dis. 213 (4), 579-583 (2016) [26] Wu *et al.* ISME J 10 (3), 609-620 (2016) [27]
KU182964.1	JTMC15	*Rhinolophus ferrumequinum*	Xu *et al.* Virol Sin 31 (1), 69-77 (2016) [29]
KP886808.1	YNLF_31C	*Rhinolophus ferrumequinum*	*Journal information is not available in the GenBank record*
KF569996.1	LYRa11	*Rhinolophus affinis*	He *et al.* J. Virol. 88 (12), 7070-7082 (2014) [30]
KC881006.1	Rs3367	*Rhinolophus sinicus*	Ge *et al.* Nature 503, 535-538 (2013) [31]
DQ412043.1	Rm1	*Rhinolophus macrotis*	Li *et al.* Science 310 (5748), 676-679 (2005) [32]
DQ412042.1	Rf1	*Rhinolophus ferrumequinum*	Li *et al.* Science 310 (5748), 676-679 (2005) [32]
GU190215.1	BtCoV/BM48-31/BGR/2008	*Rhinolophus blasii*	Drexler *et al.* J. Virol. 84 (21), 11336-11349 (2010) [33]
GQ153547.1	HKU3-12	*Rhinolophus sinicus*	Lau *et al.* J. Virol. 84 (6), 2808-2819 (2010) [34]
GQ153543.1	HKU3-8	*Rhinolophus sinicus*	Lau *et al.* J. Virol. 84 (6), 2808-2819 (2010) [34]
GQ153541.1	HKU3-6	*Rhinolophus sinicus*	Lau *et al.* J. Virol. 84 (6), 2808-2819 (2010) [34]
FJ588686.1	Rs672	*Rhinolophus sinicus*	Yuan *et al.* J. Gen. Virol. 91 (PT 4), 1058-1062 (2010) [35]
DQ071615.1	Rp3	*Rhinolophus pearsoni*	Li *et al.* Science 310 (5748), 676-679 (2005) [32]
AY304488.1	SZ16	*Paguma larvata*	Guan *et al.* Science 302 (5643), 276-278 (2003) [36]
DQ648856.1	BtCoV/273/2005	*Rhinolophus ferrumequinum*	Tang *et al.* J. Virol. 80 (15), *7481-7490* (2006) [37]
AY572034.1	civet007	Palm civet (species unspecified)	Wang *et al.* Emerging Infect. Dis. 11 (12), 1860-1865 (2005) [5]
AY502924.1	TW11	*Homo sapiens*	Yeh *et al.* Proc. Natl. Acad. Sci. U.S.A. 101 (8), 2542-2547 (2004) [38]
AY613948.1	PC4_13	Palm civet (species unspecified)	Song *et al.* Proc. Natl. Acad. Sci. U.S.A. 102 (7), 2430-2435 (2005) [21]
AY613947.1	GZ0402	*Homo sapiens*	Song *et al.* Proc. Natl. Acad. Sci. U.S.A. 102 (7), 2430-2435 (2005) [21]
AY559095.1	Sin847	*Homo sapiens*	Vega *et al.* BMC Infect. Dis. 4, 32 (2004) [39]
KF294457.1	Longquan-140	*Rhinolophus monoceros*	*Journal information is not available in the GenBank record*
DQ648857.1	BtCoV/279/2005	*Rhinolophus macrotis*	Tang *et al.* J. Virol. 80 (15), 7481-7490 (2006) [37]

Sequences were published previously[18–39].

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Reference genome sequence data were downloaded from GenBank, Virological.org and GISAID using the web interface.
Data analysis	Software used: CLC Genomic Workbench v9.0, BLAST v2.3.0+, BWA v0.7.13, MEGAHIT v1.1.3, MAFFT v7.273, PhyML v3.1, Simplot v3.5.1

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Data that support the findings of this study have been deposited in GISAID database with accession numbers EPI_ISL_410538 - EPI_ISL_410544 and to the SRA database under BioProject number PRJNA606875, and are available in the supplementary information file of this paper.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We screened all relevant pangolin samples that are available to us in the study period. Among the 43 Guangxi pangolin samples (18 pangolin individuals), 6 samples (5 pangolin individuals) were found with SARS-CoV-2 related coronavirus by sequencing. Among the 5 Guangdong pangolin samples, 1 was found with SARS-CoV-2 related coronavirus by sequencing. All these coronaviruses shared >99.7% genomic similarity to either some of them among themselves or the coronavirus found in previous study. Therefore, such sample size is sufficient for the discovery of SARS-CoV-2 related coronavirus in the pangolins in our conditions.
Data exclusions	No data were excluded.
Replication	qPCR was also applied on the same sets of samples that have been examined by metatranscriptomic sequencing, as to verify the presence of pangolin coronavirus sequence indicated by sequencing.
Randomization	There was no separation of experimental groups in the study, hence no randomization.
Blinding	There was no separation of experimental groups in the study, hence no blinding.

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Materials & experimental systems

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☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero E6 cells from ATCC.
Authentication	All Vero E6 cells were from ATCC with authentication. The authentication was performed by morphology check under microscopes and growth curve analysis.
Mycoplasma contamination	We confirm that all cells were tested as mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	No laboratory animals were involved in the study.
Wild animals	Thirty-five (18+12+5) pangolins were seized during routine anti-smuggling operations, and unfortunately dead for unknown reason in the rescue centre. Samples were then collected from them.
Field-collected samples	No field-collected samples were involved in the study.
Ethics oversight	The animals were rescued and treated by the Guangxi Zhuang Autonomous Region Terrestrial Wildlife Medical-aid and Monitoring Epidemic Diseases Research Center under the ethics approval (wild animal treatment regulation No. [2011] 85). The samples were collected following the procedure guideline (Pangolins Rescue Procedure, November, 2016).

Note that full information on the approval of the study protocol must also be provided in the manuscript.