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Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins

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The ongoing outbreak of viral pneumonia in China and beyond is associated with a novel coronavirus, SARS-CoV-2¹. 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 infection². Although bats are likely reservoir hosts for SARS-CoV-2, the identity of any intermediate host that might 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 to SARS-CoV-2 in the receptor-binding domain. 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 novel 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 coronavirus¹, which was later named SARS-CoV-2. Case numbers grew rapidly from 27 in December 2019 to 64,473 globally as of 14 February 2020³, such that the WHO have declared a public health emergency. Many of the early cases were linked to Huanan seafood market in Wuhan city, Hubei province, from where the probable zoonotic source is speculated to originate². Currently, only environmental samples taken from the market have been reported as positive for SARS-CoV-2 by the China CDC⁴. However, as similar wet markets were implicated in the SARS outbreak of 2002-2003⁵, 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 prior to the outbreak⁴. 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 closely related to SARS-CoV-2 sampled from a *Rhinolophus affinis* bat in Yunnan in 2013 has now been identified⁶, similar viruses have not yet been detected in other wildlife species. Here, we present the identification of 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 the most illegally trafficked mammal: they are used as both a food source and their scales are utilized in traditional Chinese

medicine. A number of pangolin species now are regarded as critically endangered on the International Union for Conservation of Nature Red List of Threatened Species. We received frozen tissue (lungs, intestine, blood) samples collected from 18 Malayan pangolins (*Manis javanica*) during August 2017-January 2018. These pangolins were obtained during anti-smuggling operations performed by Guangxi Customs. Strikingly, high-throughput sequencing of their RNA revealed the presence of coronaviruses in six (two lung, two intestine, one lung-intestine mix, one blood from five individual pangolins; Extended Data Table 1) of 43 samples. 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/PIE, GX/P2V, GX/P3B, GX/P4L, GX/P5E and GX/PSL - that fall into the SARS-CoV-2 lineage (within the genus *Betacoronavirus* of the *Coronaviridae*) in a phylogenetic analysis (Figure 1b). The genome sequence of the virus isolate (GX/P2V) has very high similarity (99.83-99.92%) to the five sequences obtained through the metagenomic sequencing of the raw samples, and all have similar genomic organizations to SARS-CoV-2, with eleven predicted open reading frames (Figure 1a; Extended Data Table 2; two are overlapping ORFs). We were also able to successfully isolate the virus using the Vero E6 cell line (Extended Data Figure 1). Based on these new genome sequences, we designed primers for qPCR detection to confirm that the raw samples were positive for the coronavirus. We conducted further qPCR testing on another batch of archived pangolin samples collected between May-July 2018. Among the 19 samples (nine intestine tissues,

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ten lung tissues) tested from 12 animals, three lung tissue samples from three individual pangolins were coronavirus positive.

In addition to the animals from Guangxi, 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, 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 21,505bp (denoted as GD/P2S), representing approximately 72% of the SARS-CoV-2 genome. Importantly, this virus sequence, obtained from a pangolin scale sample, may in fact be derived from contaminants of other infected tissues. Notably, another study of diseased pangolins in Guangdong performed in 2019 also identified viral contigs from lung samples that were similarly related to SARS-CoV-2⁷. 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/PIL in the phylogeny shown in Figure 1b).

These novel 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/PIL and GDP2S) is very closely related to SARS-CoV-2 (Figure 1b; red circles). It has previously been noted that members of the subgenus *Sarbecovirus* have experienced widespread recombination⁸. In support of this, a recombination analysis (Figure 2) performed here revealed that bat coronaviruses ZC45 and ZXC21 are likely recombinants, containing genome fragments derived from multiple SARS-CoV related lineages (genome regions 2, 5, 7) as well as SARS-CoV-2 related lineages including that from pangolins (regions 1, 3, 4, 6, 8).

More notable, however, was the observation of putative recombination signals between the pangolins coronaviruses, bat coronaviruses RaTG13, and human SARS-CoV-2 (Figure 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 Figure 2a and 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, human SARS-CoV numbering⁹) and these latter two viruses have only 89.2% amino acid similarity in the RBD. Interestingly, a phylogenetic analysis of synonymous sites only from the RBD revealed that the topological position of the Guangdong pangolin is consistent with that in the remainder of the viral genome, rather than being the closest relative of SARS-CoV-2 (Figure 3b). Hence, 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 choose between these scenarios on current data. This observation is consistent with the fact that ACE2 sequence similarity is higher between humans and pangolins (84.8%) than those between humans and bats (80.8%–81.4%; *Rhinolophus* sp.) (Extended Data Table 3). The occurrence of either recombination and/or convergent evolution further highlights the role played by intermediate animal hosts in human virus emergence. Importantly, however, all 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 which may have helped facilitate its emergence and rapid spread through human populations.

To date, pangolins are the only mammals other than bats documented to be infected by a SARS-CoV-2 related coronavirus. It is striking that two

related lineages of CoVs are found in pangolins 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 with relatively small population sizes, reflecting their endangered status¹¹. Indeed, on current data it cannot be excluded that pangolins acquired their SARS-CoV-2 related viruses independently from bats or another animal host, so that their role in the emergence of human SARS-CoV-2 remains unproven. In this context it is notable that both lineages of pangolin coronaviruses were obtained from trafficked Malayan pangolins, likely originating from Southeast Asia, and there is a marked lack of knowledge of the viral diversity maintained by this animal in regions where it is indigenous. Undoubtedly, the extent of virus transmission in pangolin populations requires additional investigation. However, the repeated occurrence of infections with SARS-CoV-2 related coronaviruses in Guangxi and Guangdong provinces suggests that this animal may play an important role in the community ecology of coronaviruses.

Coronaviruses, including those related to SARS-CoV-2, are clearly 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 that their sale in wet markets should be strictly prohibited. Further surveillance on pangolins in the natural environment in China and Southeast Asia are clearly needed to understand their role in the emergence of coronaviruses and the risk of future zoonotic transmission.

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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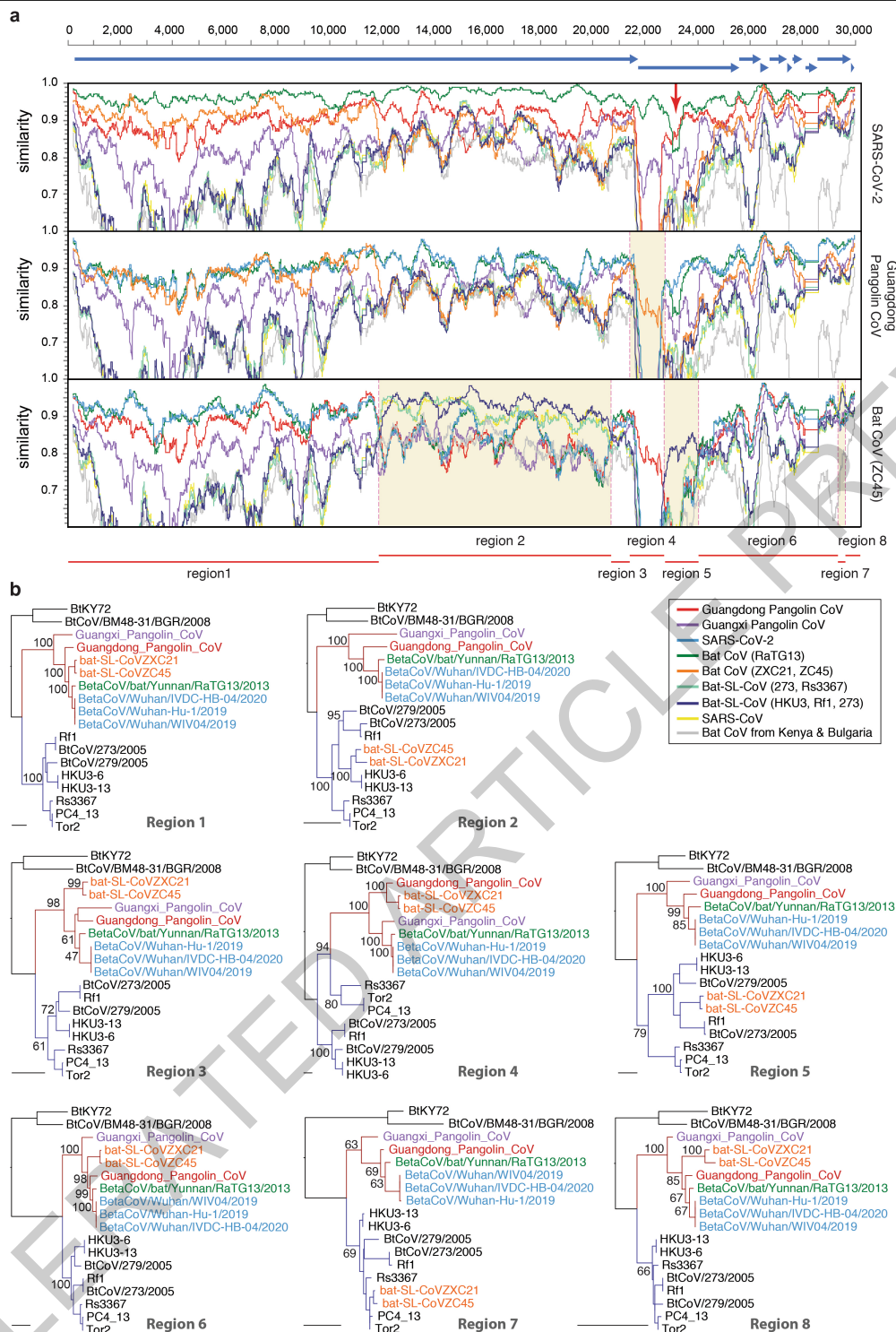


Fig. 2 | Recombination analysis. (A) Sliding window analysis of changing patterns of sequence similarity between human SARS-CoV-2, pangolin and bat coronaviruses. The potential recombination breakpoints are shown in pink dash lines, and regions separated by the breakpoints are alternatively shaded in yellow. These potential breakpoints subdivide the genomes into eight regions (regions with <200bp were omitted), indicated by the red bars at bottom for phylogenetic analysis. The name of the query sequences are shown vertically on the right of the analysis boxes. The similarities to different

reference sequences are indicated by different colors shown in the legend box at the top. Guangdong pangolin coronaviruses GD/P1L and 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. Branch supports obtained from 1,000 bootstrap replicates are shown. Branch scale bars are shown as 0.1 substitutions/site.

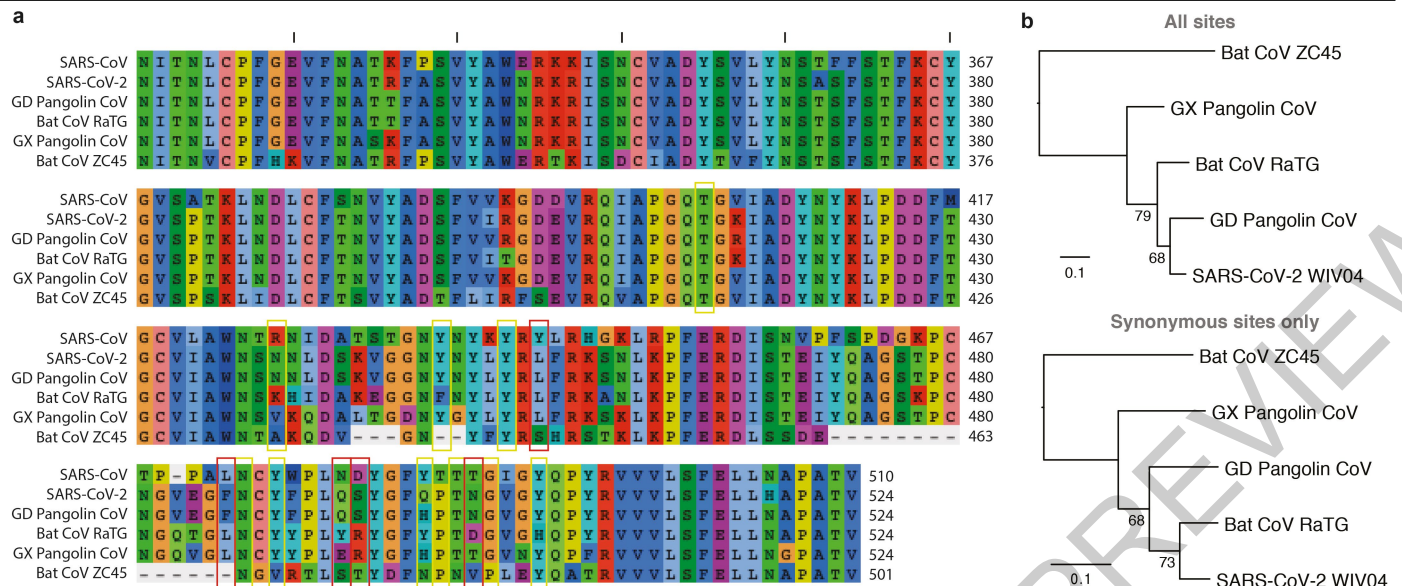


Fig. 3 | Analysis of the receptor-binding domain (RBD) sequence.

(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 with yellow boxes, following Wan et al.⁹. Note that in Guangdong pangolin sequence, the codon positions coding for amino acids

337 proline, 420 aspartic acid, 499 proline and 519 asparagine have ambiguous nucleotide compositions, resulting in possible alternative amino acids at these sites (threonine, glycine, threonine and lysine, respectively). GD: Guangdong, GX: Guangxi. (B) Phylogenetic trees of the SARS-CoV-2 related lineage estimated from the entire RBD region (upper) and synonymous sites only (lower). Branch supports obtained from 1,000 bootstrap replicates are shown.

Article

Methods

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 (*Manis 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 Figure 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, Switzerland) 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, MA, USA), 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, MA, USA). 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, utilizing 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 Figure 2a-f). Based on 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, 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, one scale) obtained in anti-smuggling operations undertaken in March 2019. RNA was extracted from all five samples (Qiagen, USA), and was subjected to high-throughput RNA sequencing on the Illumina HiSeq platform by Vision medicals, Guangdong, China. 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 v1.1.3¹³) and using reference (BWA v0.7.13¹⁴) assembly methods, using BetaCoV/Wuhan/WIV04/2019 as a reference. The contigs were combined, and approximately 72% of the coronavirus genome (21,505bp) was obtained. This sequence has about 6.6X sequencing coverage (Extended Data Figure 2g) and denoted pangolin CoV GD/P2S. This sequence has been deposited on GISAID with accession number EPI_ISL_410544.

Liu *et al.* recently published a meta-transcriptomic study of pangolins⁷ and 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 MEGAHIT¹³ and reference assembly using BWA¹⁴. These reads were then merged and curated in a pileup alignment file to obtain the consensus sequences. This combined consensus sequence is 25,753bp in length (about 86.3% of BetaCoV/Wuhan/WIV04/2019; about 6.9X coverage) and denoted pangolin CoV GD/P1L (available in the Supplementary Information Data Set). Notably, it has 66.8% overlap and a sequence identity of 99.79% with the GD/P2S sequence. Since 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 v7.273¹⁵. Maximum likelihood phylogenies were estimated using RAXML v8.2.12¹⁶ from 100 inferences, utilizing the GTRGAMMA model of nucleotide substitution with 1,000 bootstrap replicates. To investigate potential recombination events, we used SimPlot v3.5.1¹⁷ 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 Figure 3. We also examined phylogenetic clusters performed directly from the multiple sequence alignment. Maximum likelihood trees were estimated from each window extraction (i.e. genome regions 1 to 8) using RAXML as described above^{18–39}.

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 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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Author contributions Y.G. and Y.L.H. designed and supervised research. W.W., W.J.L. and L.F.L. collected samples and conducted genome sequencing. 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, X.B.N, W.Y.M.C., E.C.H. and Y.S.L. performed genome analysis and interpretation. T.T.Y.L. and E.C.H. wrote the paper. H.C.Z., Y.L.H., G.M.L and Y.G. joined the data interpretation and edited the paper.

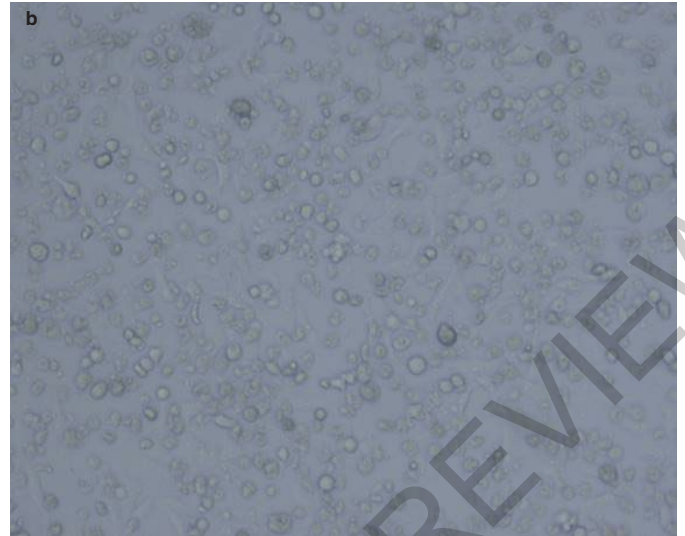
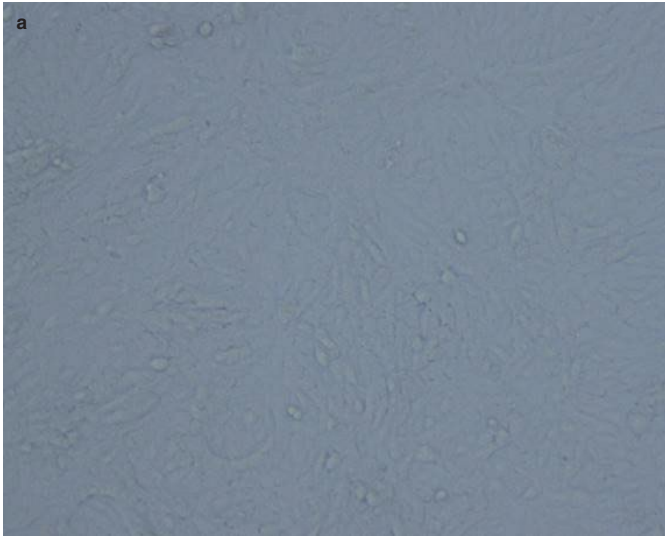
Competing interests The authors declare no competing interests.

Additional information

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Peer review information *Nature* thanks Paul Kellam, Ian Lipkin and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

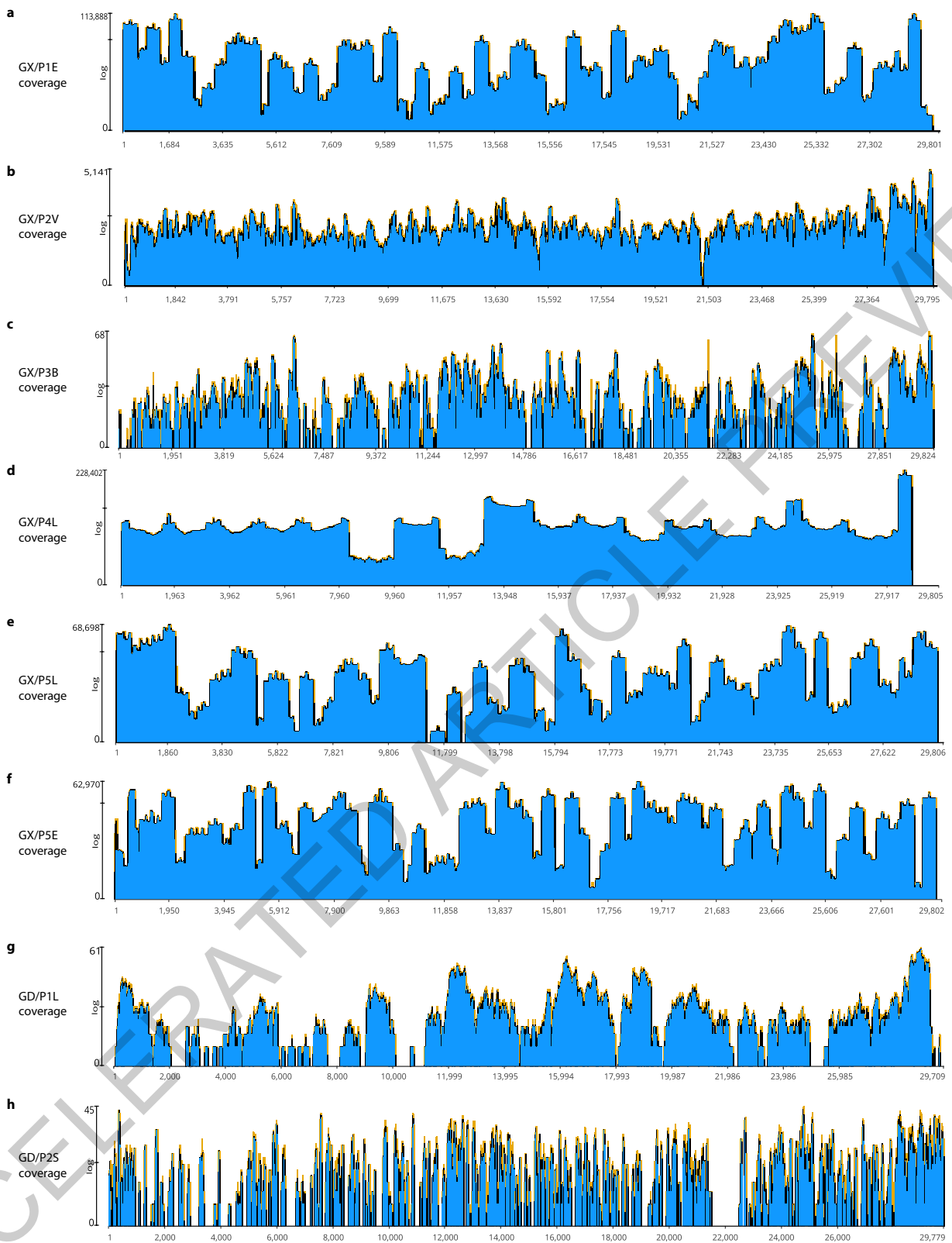
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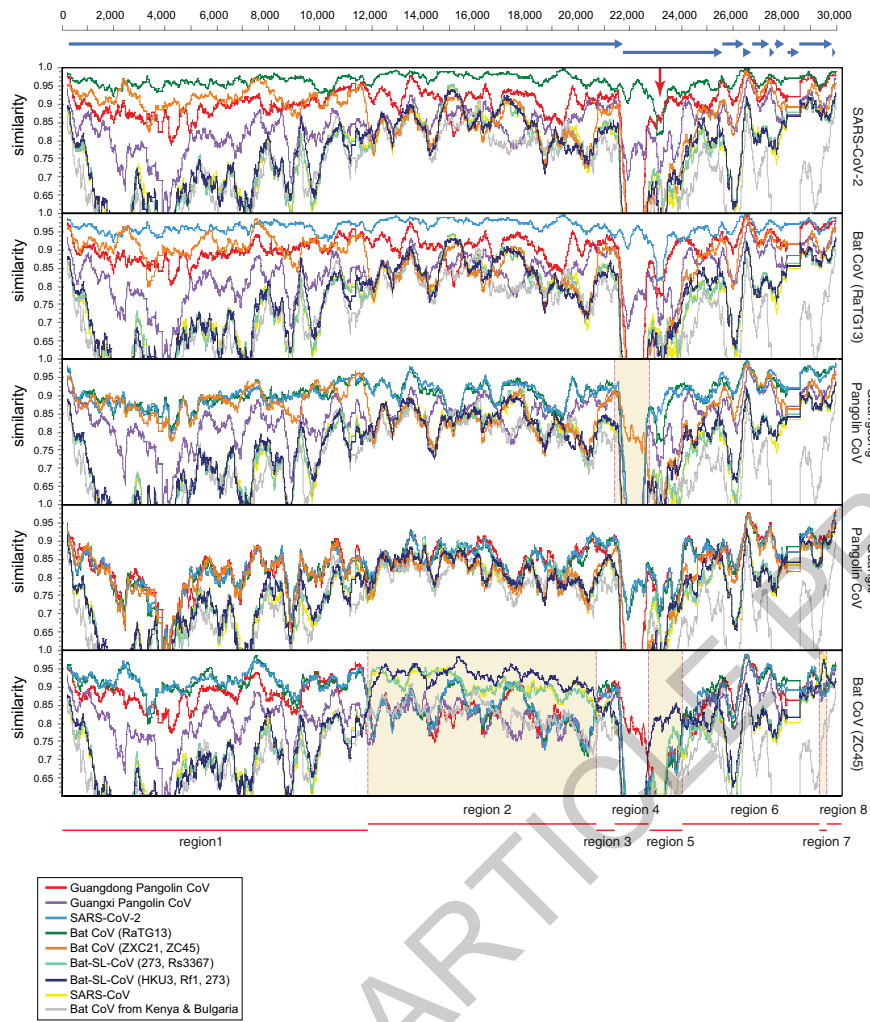
Extended Data Fig. 1 | Microscopic image of the cytopathic effect in virus isolation using Vero E6. (A) Negative control of Vero E6 cell line. (B) Cytopathic effect seen in viral culture (5 days post inoculation). The experiment was

performed two times independently in two laboratories and produced similar results.

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Extended Data Fig. 2 | Read coverage depth of each pangolin coronavirus analyzed in this study.



Extended Data Fig. 3 | Recombination analysis of all members in SARS-CoV-2 related lineage. Same legend description as Figure 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 (<https://www.ncbi.nlm.nih.gov/sra>) under BioProject PRJNA606875.

Article

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

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

* partial sequence

Extended Data Table 3 | Sequence similarity of Angiotensin-converting enzyme 2 (ACE2) amino acid sequences between humans, pangolins and bats.

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

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

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

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Extended Data Table 5 | Acknowledgement of sharing of SARS-CoV-2 genome sequences available at the Virological.org and the GISAID databases.

Accession ID	Virus name	Location	Collection date	Originating lab	Submitting lab	Authors
Virological.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

We gratefully thank the authors listed below for sharing their genomic sequences of coronaviruses analyzed in this study.

Article

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

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

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Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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Software and code

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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 Behavioural & social sciences Ecological, evolutionary & environmental sciences

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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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<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
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<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> 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).

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