Supporting information

Differential prevalence and risk factors for infection with coronaviruses in bats collected from Yunnan Province, China

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Supplementary methods

Method S1. Samples collection, bat identification and cave locations

Samples were collected from bats in 10 different locations: site A (Location: Damodan, Fumin, Kunming (KM); sampling date: 19/09/2020), site B (Qinglongshan, Jinning, KM; 23/09/2020), site C (Boluohei, Shilin, KM; 01/10/2020), site D (Caozidi, Shilin, KM; 30/09/2020), site E (Xiaoboxi, Shilin, KM; 02/10/2020), site F (Tianshengguan, Shilin, KM; 28/09/2020), site G (Tianshengguan, Shilin, KM; 29/09/2020), site H (Laolengdi, Xishan, KM; 24/09/2020), site I (Miaoxinzhai, Mengla, Xishuangbanna (XSBN); 26/11/2020) and site J (Shijiancao, Yimen, Yuxi (YX); 21/09/2020). Bats were collected using 4 bank harp-traps, as well as mistnets, though the number and distributions of these traps and nets varied depending on site specific factors. Nets were set to be ready 30 min before sunset, and left open until activity levels dropped to zero new bats in a 1 h period (typically between 9 pm to midnight). Harp traps were initially checked 30 min after sunset and then checked approximately every 30 min, whereas mistnets were monitored almost constantly. Captured bats were removed and stored in a cloth bag for processing. Species were identified based on our pre-existing analysis which had been validated through barcoding, as well as detailed comparisons of detailed physiology and acoustics [1]. Measurements were made with digital callipers Mitutoyo Absolute Series-500 (accuracy 0.01 mm) and body mass was measured with Pesola Spring Scale (Pesola®Präzisionswaagen AG) with a precision of 0.3%) and included forearm length, head-body, tibia, tail, ear height, and for rhinolophoid bats noseleaf height and width. In addition, profile photos were taken of all individuals, and tissue samples were removed from the wing membrane using a 3mm biopsy punch and stored in 99.7% ethanol.

Rectal swabs from individual bats were collected and the samples were stored in RNAlater (Thermo Fisher Scientific, MA, USA) and transported to the laboratory in dry ice, then stored at -80°C until further processing. The presence, number and identity of ectoparasites on bats were recorded during sampling in site A, site B, site C, site E, site F and site H. Baidu Coordinate Picker (https://api.map.baidu.com/lbsapi/getpoint/) was used to obtain the specific geographical coordinates for the sampling locations, and verified using satellite imagery data in ArcMap.

Method S2. Nucleic acid extraction from bat rectal swabs

The extraction was performed according to the supplied protocol (Publication number MAN0012669) with the following modifications: 2 times the volume (400 µL) of input sample solution was used for obtaining a potentially higher viral load. As such, the lysis buffer and proteinase K used were correspondingly doubled in volume. The nucleic acid was eluted with 50 µL RNase-free water (Invitrogen, MA, USA), and stored at -80°C pending further processing.

Method S3. Degenerate PCR primer design for coronaviruses detection

Coronavirus sequences were downloaded from NCBI and analyzed in Geneious R9. After sorting, protein sequences were aligned to find the conserved motif of RdRp as the target domain and 1360 RdRp sequences of coronaviruses were extracted and aligned for primer design. During the primer design, inverse degeneration was performed following the codon list [2]. The sequence up to approximately 20 bp upstream from the target was also selected as a clamp sequence to complete three universal primers for pan-coronavirus (pan-CoV) semi-nested PCR detection (during primer synthesis, the N residues in the primer sequences were replaced with hypoxanthine (I)). The sequences of the pan-CoV outer semi-nested PCR primers (target size 298 bp) were as follows: Pan-CoVs-OF: 5’-

TGTTATTGGAACAACTAAATTYTAYGGNGGNTG-3’ (position 1900 to 1933 in alignment) and Pan-CoVs-OR: 5’-GGTTGCATCACCACTACTAGTNCCNCCNGGYTT-3’ (position 2165 to 2197 in alignment); The pan-CoVs inner semi-nested PCR primer (target size 236 bp) was as follows: Pan-CoVs-IF: 5’-GTTTTGAAAATCCTATTCTTATGGGNTGGGAYTAYCC-3’ (position 1962 to 1998 in alignment), whereas the Pan-CoVs-OR was used as the reverse primer. The alignment is provided in an extended txt file. Sanger sequencing was performed with the following primers: CoVs-seq-OR: 5’-GGTTGCATCACCACTACT-3’ and CoVs-seq-IF: 5’-TGAAAATCCTATTCTTATG-3’.

Method S4. cDNA synthesis and pan-CoV semi-nested PCR detection

Random hexamers (50 μM) were used in this kit as random primers in synthesis. The protocol is as follows: reverse transcription at 30°C for 10 min, then 42°C for 45 min. Enzyme denaturation at 95°C for 5 min, before proceeding to PCR.

The pan-CoV outer PCR mix is as follows: 10 μL TAKARA Ex Taq Version 2.0 plus dye (TAKARA, Dalian, China), 1 μL Pan-CoVs-OF (10 μM) as forward primer, 1 μL Pan-CoVs-OR (10 μM) as reverse primer, 2 μL cDNA, and nuclease-free water was added to make a total reaction volume of 20 μL. The pan-CoV outer PCR protocol is as follows: Initial denaturation at 94°C for 2 min, 5 cycles (denaturation at 94°C for 30 s, annealing at 44°C for 30 s and extension at 72°C for 20 s), 30 cycles (denaturation at 94°C for 30 s, annealing at 54°C for 30 s and extension at 72°C for 20 s), followed by a final extension at 72°C for 2 min and hold at 4°C.

The pan-CoV inner PCR mix is as follows: 10 μL TAKARA Ex Taq Version 2.0 plus dye, 1 μL Pan-CoVs-IF (10 μM) as forward primer, 1 μL Pan-CoVs-OR (10 μM) as reverse primer, 0.8 μL outer PCR products, 1 μL 5% DMSO (Yeasen Biotech, Shanghai, China) and nuclease-free water was added to make a total reaction volume of 20 μL. The pan-CoV inner PCR protocol is as follows: Initial denaturation at 94°C for 2 min, 5 cycles (denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 20 s), 30 cycles (denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 20 s), followed by a final extension at 72°C for 2 min and hold at 4°C.

Method S5. DNA agarose gel electrophoresis and Sanger sequencing

1.5% agarose (Baygene, Shanghai, China) gel was in 1×TAE buffer (Sangon, Shanghai, China) for DNA electrophoresis. A concentration of 0.1% nucleic acid dye Tanon, Shanghai, China) was added to the agarose gel to visualize the DNA. 3 μL of DL5000 Marker (Tanon, Shanghai, China) was used and 6 μL of sample were loaded into each well. The DNA agarose gel electrophoresis (AGE) was run at 110 V for 40 min. After the AGE run was complete, a gel image was taken to identify a 236 bp band at the expected targeted size. When the band of the expected size was present, 3 tubes for PCR were prepared for each positive template, and the AGE was performed again as described above. The target bands were excised under UV light for Sanger sequencing (Tsingke, Beijing, China). Bidirectional sequencing with reverse primer (CoVs-seq-OR) and forward primers (CoVs-seq-IF) were performed to enable nested PCR for all positive templates.

Method S6. Identity verification of bats with SARSr-CoVs

Using two pairs of primers (inner and outer) for nested PCR amplification, bat species were identified by molecular barcoding, targeting the conserved gene cytochrome c oxidase subunit I (COI). The primer (Tsingke) sequences [3] are as follows: Outer nested PCR primers: BEGLCOIf: 5’- GGYGCYTGAGCHGGWATAGT-3’ and BEGLCOIr: 5’- ARRATDGGRTCYCCYCCTCC-3’. The reaction consists of 10 μL

TAKARA Ex Taq Version 2.0 plus dye (TAKARA, Dalian, China), 1 µL BEGLCOIf (10 µM) as forward primer, 1 µL BEGLCOIr (10 µM) as reverse primer, 1.5 µL cDNA and nuclease-free water to a total volume of 20 µL. The reaction conditions are: Initial denaturation at 94°C for 2 min, 35 cycles (denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 50 s), final extension at 72°C for 2 min and hold at 4°C. Inner nested PCR primers: SFF_145f: 5’-GTHACHGCYCAYGCHTTYGTAATAAT-3’ and SFF_351r: 5’-CTCCWGCRTGDGCWAGRTTTCC-3’. The reaction consists of 10 µL TAKARA Ex Taq Version 2.0 plus dye, 1 µL SFF_145f (10 µM) as forward primer, 1 µL SFF_351r (10 µM) as reverse primer, 0.5 µL outer PCR products, 1 µL 5% DMSO (Yeasen Biotech, Shanghai, China) and nuclease-free water to a total volume of 20 µL. The reaction conditions are: Initial denaturation at 94°C for 2 min, 35 cycles (denaturation at 94°C for 30 s, annealing at 53°C for 30 s and extension at 72°C for 20 s), final extension at 72°C for 2 min and hold at 4°C.

Human coronavirus 229E (HCoV-229E) and a null template control were utilized as positive and negative controls, respectively, in each batch of nested PCR and agarose gel electrophoresis.

Supplementary figures

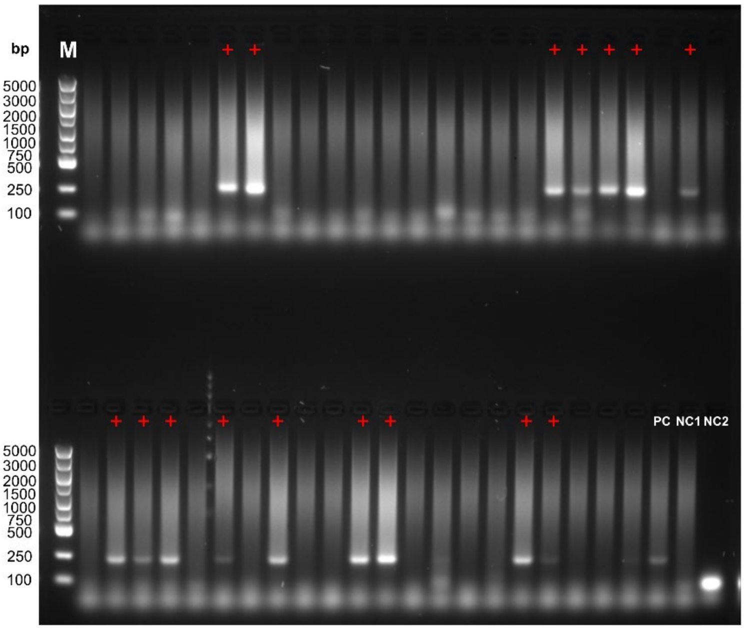


Figure S1. DNA agarose electrophoresis (AGE) gel of the pan-CoVs inner semi-nested PCR. The AGE gel image displays the expected target band size (236 bp) for CoV positives (+). From the left to the right, shows C-24A/C-26A/C-40A/C-44A/C-45A/C-46A/C-49A (upper panel) and C-56A/C-57A/C-58A/C-61A/C-64A/C-69A/C-70A/C-80A/C-84A (lower panel) respectively. M, DL5000 marker; PC, positive control; NC1, negative control in the pan-CoVs outer semi-nested PCR; NC2, negative control in the pan-CoVs inner semi-nested PCR.

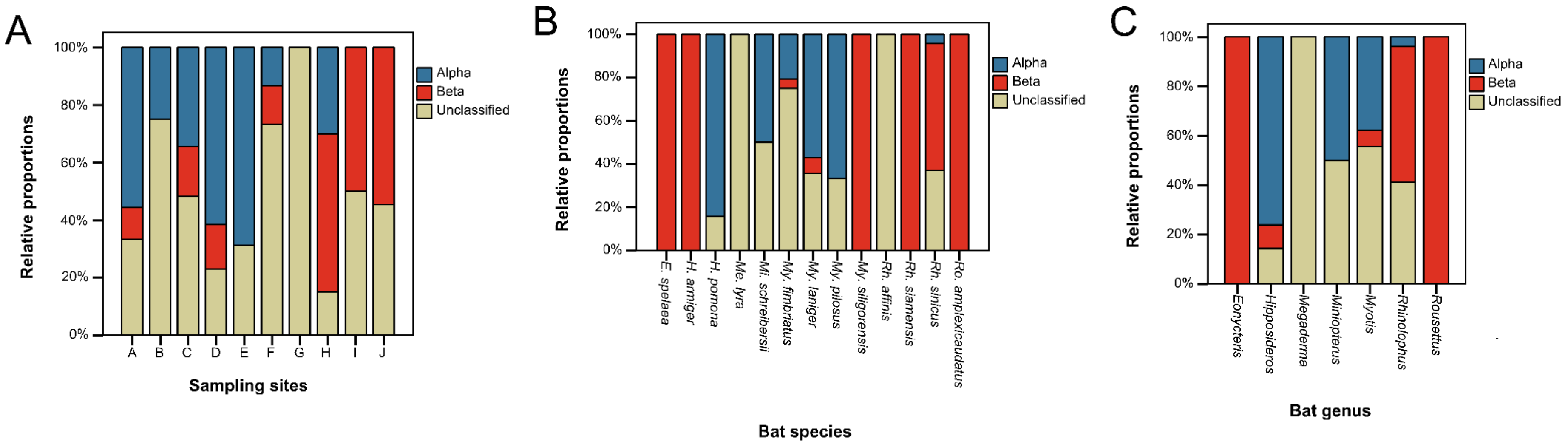
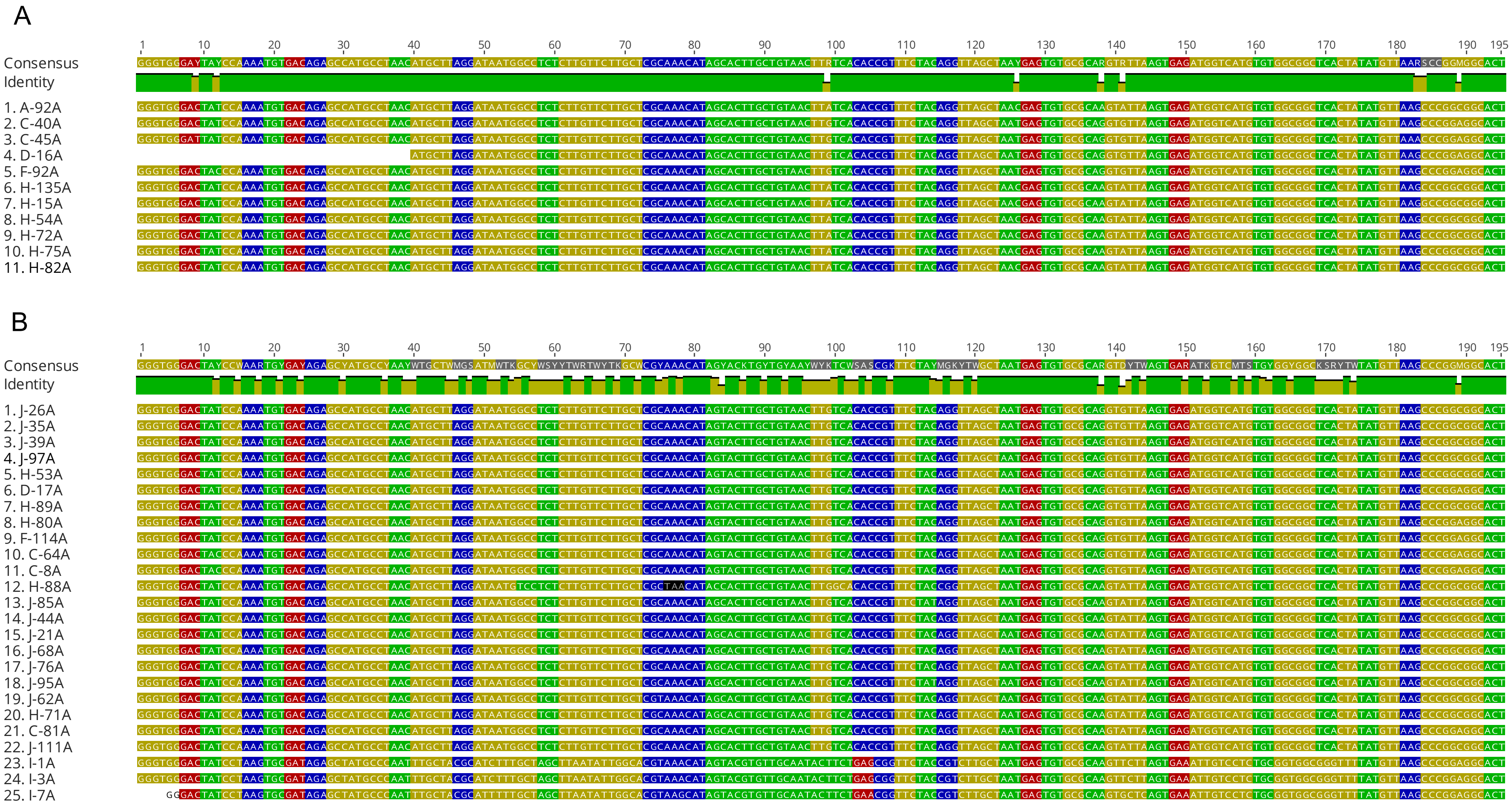


Figure S2 (A) Differential prevalence of coronaviruses (α-CoV, β-CoV and unclassified CoV) in ten sampling sites (A-J). (B) Differential prevalence of CoVs in 13 bat species. The x-axis indicates bat genus (C) and bat species (B). (C) Differential prevalence of CoVs in 7 bat genra. The y-axis indicates the number of CoV-positives scaled to percentage. The yellow column indicates the number of unclassified CoV positives. The red column indicates the number of β-CoV positives. The blue column indicates the number of α-CoV positives.


A
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140
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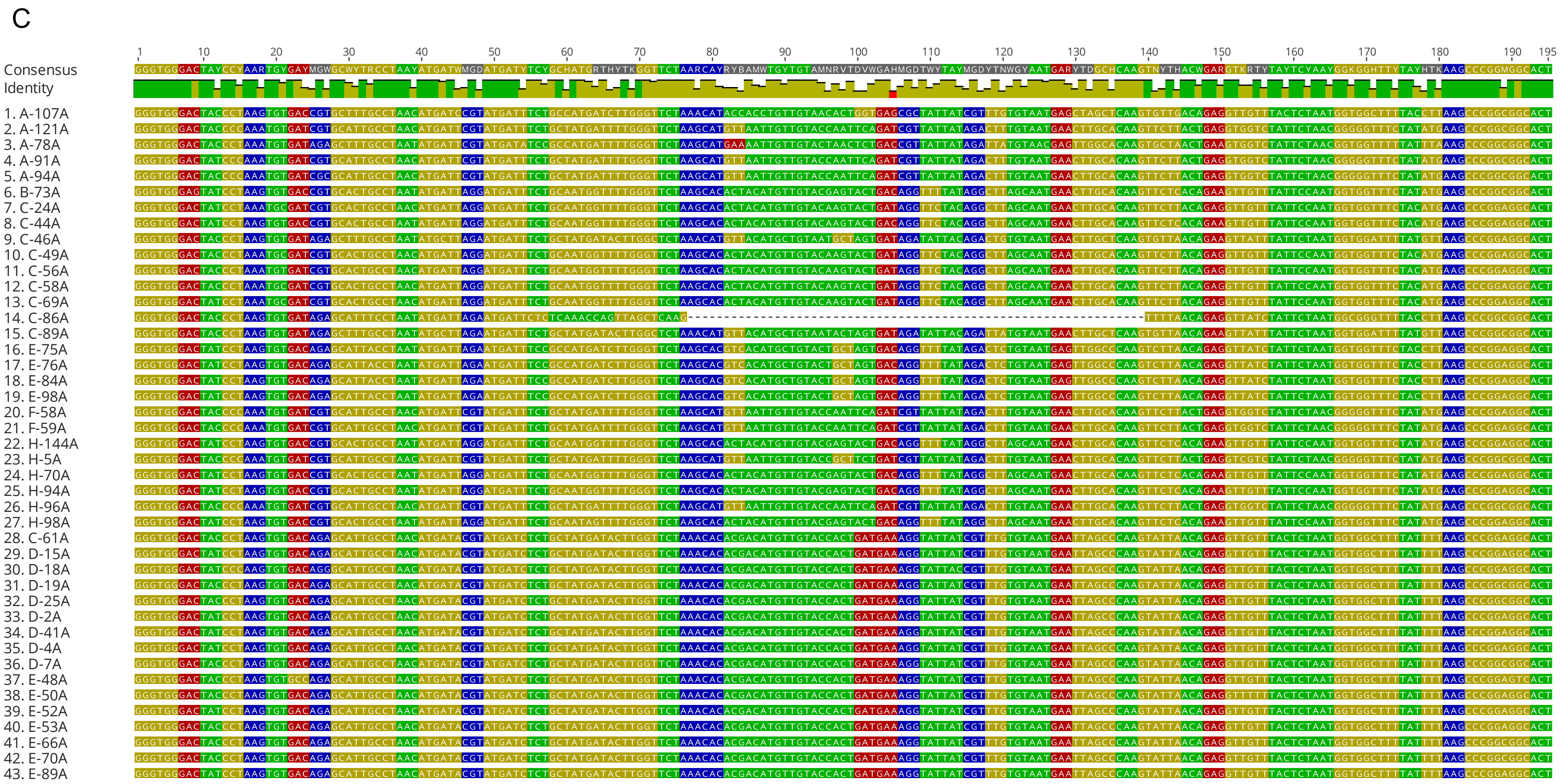
170
180
190
195
Consensus
Identity
1. A-92A
2. C-40A
3. C-45A
4. D-16A
5. F-92A
6. H-135A
7. H-15A
8. H-54A
9. H-72A
10. H-75A
11. H-82A
B
Consensus
Identity
1. J-26A
2. J-35A
3. J-39A
4. J-97A
5. H-53A
6. D-17A
7. H-89A
8. H-80A
9. F-114A
10. C-64A
11. C-8A
12. H-88A
13. J-85A
14. J-44A
15. J-21A
16. J-68A
17. J-76A
18. J-95A
19. J-62A
20. H-71A
21. C-81A
22. J-111A
23. I-1A
24. I-3A
25. I-7A


C
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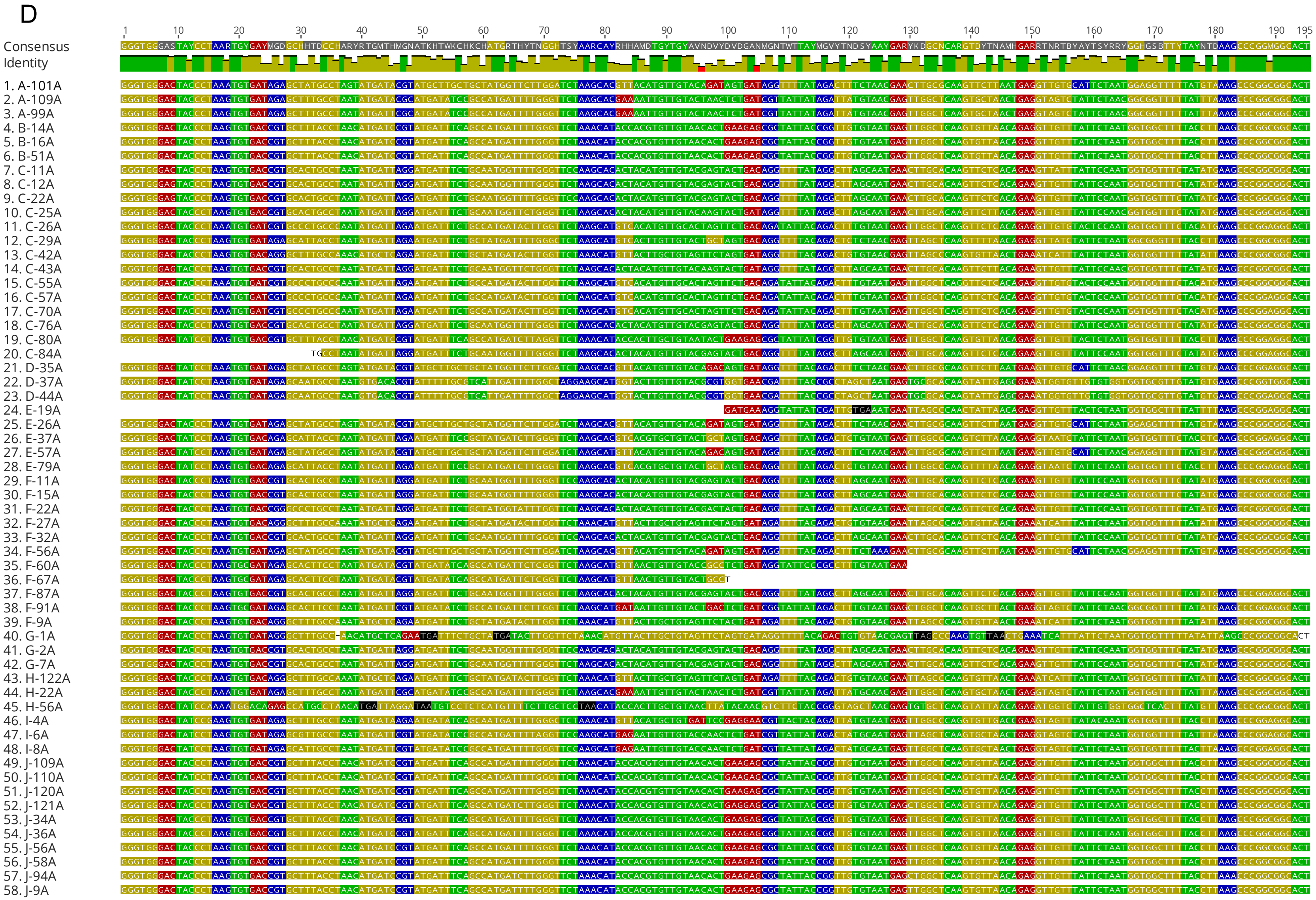
160
170
180
190
195
Consensus
Identity
1. A-107A
2. A-121A
3. A-78A
4. A-91A
5. A-94A
6. B-73A
7. C-24A
8. C-44A
9. C-46A
10. C-49A
11. C-56A
12. C-58A
13. C-69A
14. C-86A
15. C-89A
16. E-75A
17. E-76A
18. E-84A
19. E-98A
20. F-58A
21. F-59A
22. H-144A
23. H-5A
24. H-70A
25. H-94A
26. H-96A
27. H-98A
28. C-61A
29. D-15A
30. D-18A
31. D-19A
32. D-25A
33. D-2A
34. D-41A
35. D-4A
36. D-7A
37. E-48A
38. E-50A
39. E-52A
40. E-53A
41. E-66A
42. E-70A
43. E-89A


D
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100
110
120
130
140
150
160
170
180
190
195
Consensus
Identity
1. A-101A
2. A-109A
3. A-99A
4. B-14A
5. B-16A
6. B-51A
7. C-11A
8. C-12A
9. C-22A
10. C-25A
11. C-26A
12. C-29A
13. C-42A
14. C-43A
15. C-55A
16. C-57A
17. C-70A
18. C-76A
19. C-80A
20. C-84A
21. D-35A
22. D-37A
23. D-44A
24. E-19A
25. E-26A
26. E-37A
27. E-57A
28. E-79A
29. F-11A
30. F-15A
31. F-22A
32. F-27A
33. F-32A
34. F-56A
35. F-60A
36. F-67A
37. F-87A
38. F-91A
39. F-9A
40. G-1A
41. G-2A
42. G-7A
43. H-122A
44. H-22A
45. H-56A
46. I-4A
47. I-6A
48. I-8A
49. J-109A
50. J-110A
51. J-120A
52. J-121A
53. J-34A
54. J-36A
55. J-56A
56. J-58A
57. J-94A
58. J-9A

Figure S3. Alignment of 195bp targeted RdRp sequences of coronaviruses from 137 CoV-positive individuals. (A) Alignment for 11 SARSr-CoV positives. (B) Alignment for 25 β-CoV positives, excluding the 11 SARSr-CoVs. (C) Alignment for 43 α-CoV positives. (D) Alignment for 58 unclassified CoV positives. All the sequences are available by the accessions in NMDC attached in a txt file ‘sequences ID and accessions’ and ‘137 targeted RdRp sequences’ in Supporting Information.

Supplementary tables

Table S1. Coronavirus prevalence in rectal swabs from bats in Yunnan province, China.

Sampling sites / Species[#]	No. of CoV-positive samples/total no. of samples; CoV-prevalence of sampling site[a]:										
	Kunming								**Xishuangbanna**	**Yuxi**	**All locations**
Species[#]	**A**	**B**	**C#**	**D#**	**E**	**F**	**G^**	**H**	**I^**	**J#**	
*E. spelaea^*	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	2/2; 100.0%^	0/0; NA	2/2; 100.0%^
H. armiger	0/0; NA	0/0; NA	1/5; 20.0%	0/0; NA	0/12; 0.0%	0/1; 0.0%	0/0; NA	1/4; 25.0%	0/0; NA	0/5; 0.0%	2/27; 7.4%
H. pomona	0/1; 0.0%	0/0; NA	1/1; 100.0%^	10/44; 22.7%	8/48; 16.7%	0/2; 0.0%	0/0; NA	0/0; NA	0/0; NA	0/0; NA	19/96; 19.8%
I. io	0/1; 0.0%	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/1; 0.0%
*Me. lyra^*	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	1/2; 50.0%^	0/0; NA	1/2; 50.0%
Mi. schreibersii#	6/7; 85.7%^	0/0; NA	0/0; NA	0/0; NA	0/0; NA	5/34; 14.7%	0/0; NA	3/6; 50.0%^	2/3; 66.7%^	0/10; 0.0%	16/60; 26.7%#
My. fimbriatus	0/0; NA	1/9; 11.1%	9/20; 45.0%#	0/0; NA	0/0; NA	6/21; 28.6%#	3/5; 60.0%^	5/49; 10.2%	0/0; NA	0/0; NA	24/104; 23.1%
My. laniger	0/0; NA	0/37; 0.0%	14/46; 30.4%#	0/0; NA	0/0; NA	0/3; 0.0%	0/1; 0.0%	0/1; 0.0%	0/0; NA	0/4; 0.0%	14/92; 15.2%
My. pilosus	0/0; NA	0/0; NA	0/0; NA	0/0; NA	6/19; 31.6%#	0/6; 0.0%	0/0; NA	0/1; 0.0%	0/0; NA	0/0; NA	6/26; 23.1%
*My. siligorensis^*	0/0; NA	0/1; 0.0%	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	1/1; 100.0%^	1/2; 50.0%
My. sp.	0/3; 0.0%	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/3; 0.0%
Rh. affinis	0/22; 0.0%	0/0; NA	0/1; 0.0%	1/2; 50.0%^	2/23; 8.7%	1/29; 3.4%	0/2; 0.0%	0/0; NA	0/0; NA	0/17; 0.0%	4/96; 4.2%
Rh. pusillus	0/0; NA	0/0; NA	0/2; 0.0%	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/1; 0.0%	0/0; NA	0/5; 0.0%	0/8; 0.0%
Rh. rex	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/3; 0.0%	0/0; NA	0/0; NA	0/0; NA	0/6; 0.0%	0/9; 0.0%
Rh. siamensis	0/0; NA	0/0; NA	0/6; 0.0%	0/0; NA	0/0; NA	0/0; NA	0/0; NA	1/8; 12.5%	0/0; NA	0/11; 0.0%	1/25; 4.0%
Rh. sinicus#	3/11; 27.3%^	3/22; 13.6%	4/8; 50.0%^	2/2; 100.0%^	0/0; NA	3/25; 12.0%	0/0; NA	10/66; 15.2%	0/1; 0.0%	21/27; 77.8%#	46/162; 28.4%#
*Ro. amplexicaudatus^*	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	0/0; NA	1/1; 100.0%^	0/0; NA	1/1; 100.0%^
All species	9/45; 20.0%	4/69; 5.8%	29/89; 32.6%#	13/48; 27.1%#	16/102; 15.7%	15/124; 12.1%	3/8; 37.5%^	20/136; 14.7%	6/9; 66.7%^	22/86; 25.6%#	137/716; 19.1%

[a]NA, no bats collected. #, species belong to the families: Pteropodidae (*E. spelaea* and *Ro. amplexicaudatus*), Hipposideridae (*H. armiger* and *H. pomona*), Vespertilionidae (*I. io, My. fimbriatus, My. laniger, My. pilosus, My. siligorensis* and *My. sp.*), Miniopteridae (*Mi. schreibersii*), Megadermatidae (*Me. lyra*), and Rhinolophidae (*Rh. affinis, Rh. pusillus, Rh. rex, Rh. siamensis* and *Rh. sinicus*). Notable samples are indicated by # if there are over 15 bats with an incidence of over 25% and ^ indicates a high percentage (27%-100%) that may not be representative, due to small sample size.

Table S2. Sex of sampled bats as distributed across species.

Bat Species#	Bat sex			Total
	Female	Male	Unidentified sex	
E. spelaea	1	1	0	2
H armiger	19	8	0	27
H. pomona	56	38	2	96
I. io	NA	NA	1	1
Me. lyra	NA	2	0	2
Mi. schreibersii	31	20	9	60
My. fimbriatus	54	49	1	104
My. laniger	50	42	0	92
My. pilosus	4	22	0	26
My. siligorensis	1	1	0	2
My. sp.	NA	NA	3	3
Rh. affinis	47	26	23	96
Rh. pusillus	5	3	0	8
Rh. rex	6	3	0	9
Rh. siamensis	15	8	2	25
Rh. sinicus	87	63	12	162
Ro. amplexicaudatus	NA	1	0	1
Total	376	287	53	716

NA, no bats collected or no value. #, species belong to families: Pteropodidae (*E. spelaea* and *Ro. amplexicaudatus*), Hipposideridae (*H. armiger* and *H. pomona*), Vespertilionidae (*I. io, My. fimbriatus, My. laniger, My. pilosus, My. siligorensis* and *My. sp.*), Miniopteridae (*Mi. schreibersii*), Megadermatidae (*Me. lyra*), and Rhinolophidae (*Rh. affinis, Rh. pusillus, Rh. rex, Rh. siamensis* and *Rh. sinicus*).

Table S3. BLASTn results for CoV-positive samples.

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
1	D-19A	*H. pomona*	F	195	94.9	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
2	D-25A	*H. pomona*	F	197	96.72	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
3	D-7A	*H. pomona*	F	195	94.9	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
4	E-66A	*H. pomona*	F	195	94.9	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
5	E-70A	*H. pomona*	F	195	94.9	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
6	C-61A	*H. pomona*	M	195	94.9	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
7	E-48A	*H. pomona*	M	195	96.17	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
8	E-50A	*H. pomona*	F	195	94.39	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
9	E-52A	*H. pomona*	M	195	94.39	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
10	E-53A	*H. pomona*	M	195	94.9	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
11	E-89A	*H. pomona*	M	196	94.42	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
12	D-18A	*H. pomona*	F	195	93.88	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
13	D-41A	*H. pomona*	M	195	94.39	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
14	D-4A	*H. pomona*	F	195	94.39	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
15	D-15A	*H. pomona*	M	195	94.39	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*
16	D-2A	*H. pomona*	M	195	94.39	MN611523.1	*Alphacoronavirus*	*Decacovirus*	*H. pomona bat coronavirus HKU10-related*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
17	A-121A	*Mi. schreibersii*	NA	195	97.45	KJ473800.1	*Alphacoronavirus*	*Minunacovirus*	*Miniopterus bat coronavirus HKU8*
18	A-91A	*Mi. schreibersii*	NA	195	96.94	KJ473800.1	*Alphacoronavirus*	*Minunacovirus*	*Miniopterus bat coronavirus HKU8*
19	A-94A	*Mi. schreibersii*	NA	195	96.94	KJ473800.1	*Alphacoronavirus*	*Minunacovirus*	*Miniopterus bat coronavirus HKU8*
20	H-5A	*Mi. schreibersii*	F	195	95.41	KJ473800.1	*Alphacoronavirus*	*Minunacovirus*	*Miniopterus bat coronavirus HKU8*
21	H-96A	*Mi. schreibersii*	F	195	97.45	KJ473800.1	*Alphacoronavirus*	*Minunacovirus*	*Miniopterus bat coronavirus HKU8*
22	F-58A	*Mi. schreibersii*	M	195	97.45	KJ473800.1	*Alphacoronavirus*	*Minunacovirus*	*Miniopterus bat coronavirus HKU8*
23	F-59A	*Mi. schreibersii*	F	195	97.45	KJ473800.1	*Alphacoronavirus*	*Minunacovirus*	*Miniopterus bat coronavirus HKU8*
24	C-89A	*My. laniger*	M	195	97.73	KU973689.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
25	B-73A	*My. fimbriatus*	M	195	94.62	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
26	C-24A	*My. laniger*	F	195	96.24	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
27	C-44A	*My. laniger*	F	195	96.24	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
28	C-46A	*My. laniger*	F	195	98.3	KU973689.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
29	C-49A	*My. laniger*	F	195	95.7	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
30	C-56A	*My. laniger*	M	195	96.24	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
31	C-58A	*My. laniger*	F	195	95.7	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
32	C-69A	*My. laniger*	M	195	96.24	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
33	H-70A	*Rh. sinicus*	F	195	94.62	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
34	H-144A	*My. fimbriatus*	M	195	94.62	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
35	H-94A	*My. fimbriatus*	M	195	94.62	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
36	H-98A	*My. fimbriatus*	M	196	94.12	MZ081397.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Alphacoronavirus sp.*
37	E-75A	*My. pilosus*	M	195	98.36	DQ249224.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Bat coronavirus HKU6*
38	E-76A	*My. pilosus*	M	195	98.36	DQ249224.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Bat coronavirus HKU6*
39	E-84A	*My. pilosus*	M	195	98.36	DQ249224.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Bat coronavirus HKU6*
40	E-98A	*My. pilosus*	M	195	98.36	DQ249224.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Bat coronavirus HKU6*
41	A-78A	*Mi. schreibersii*	NA	196	98.37	KJ473804.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*BtMf-AlphaCoV/HeN2013-a*
42	A-107A	*Rh. sinicus*	NA	195	96.43	MK211373.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Coronavirus BtRs-AlphaCoV/YN2018*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
43	C-86A	*My. fimbriatus*	F	132	89.74	KP895494.1	*Alphacoronavirus*	*unclassified Alphacoronavirus*	*Myotis Bat Alphacoronavirus strain YNXY_46C*
44	I-1A	*Ro. amplexicaudatus*	M	195	96.94	KU762338.1	*Betacoronavirus*	*Nobecovirus*	*Rousettus bat coronavirus GCCDC1*
45	I-3A	*E. spelaea*	F	195	96.94	KU762338.1	*Betacoronavirus*	*Nobecovirus*	*Rousettus bat coronavirus GCCDC1*
46	I-7A	*E. spelaea*	M	191	96.34	KU762337.1	*Betacoronavirus*	*Nobecovirus*	*Rousettus bat coronavirus GCCDC1*
47	H-15A	*Rh. cf. thomasi*	F	195	98.91	KY417149.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*
48	A-92A	*Rh. sinicus*	NA	195	98.91	KY417149.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*
49	H-54A	*Rh. sinicus*	F	195	98.91	KY417149.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*
50	H-72A	*Rh. sinicus*	M	195	98.91	KY417149.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
									syndrome-related coronavirus
51	H-75A	*Rh. siamensis*	F	195	98.91	KY417149.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*
52	F-92A	*Rh. sinicus*	F	195	98.91	FJ588686.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*
53	H-135A	*Rh. sinicus*	F	195	98.91	KY417149.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*
54	H-82A	*Rh. sinicus*	F	195	98.91	KY417149.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*
55	C-40A	*Rh. sinicus*	M	195	99.45	FJ588686.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*
56	C-45A	*My. laniger*	M	195	99.46	KY417145.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
57	D-16A	*Rh. sinicus*	M	156	100	KY417145.1	*Betacoronavirus*	*Sarbecovirus*	*Severe acute respiratory syndrome-related coronavirus*
58	J-26A	*Rh. sinicus*	F	195	98.91	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
59	J-35A	*Rh. sinicus*	F	195	98.91	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
60	J-39A	*Rh. sinicus*	F	195	98.91	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
61	J-97A	*Rh. sinicus*	F	195	98.91	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
62	J-85A	*Rh. sinicus*	F	195	98.91	MK211377.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018C*
63	J-44A	*Rh. sinicus*	M	195	98.91	MK211378.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018D*
64	J-111A	*Rh. sinicus*	M	195	98.91	MK211378.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018D*
65	J-21A	*Rh. sinicus*	M	195	98.91	MK211378.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018D*
66	J-68A	*Rh. sinicus*	M	195	98.91	MK211378.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018D*
67	J-76A	*Rh. sinicus*	M	195	98.91	MK211378.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018D*
68	J-95A	*Rh. sinicus*	M	195	98.91	MK211377.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018C*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
69	J-62A	*My. siligorensis*	M	195	98.36	MK211378.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018D*
70	H-53A	*Rh. sinicus*	F	195	98.91	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
71	H-71A	*H. armiger*	F	195	98.91	MK211378.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018D*
72	F-114A	*Rh. sinicus*	M	195	98.91	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
73	H-88A	*Rh. sinicus*	M	196	94.57	MK211378.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018D*
74	H-89A	*Rh. sinicus*	F	195	98.91	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
75	C-64A	*Rh. sinicus*	F	195	98.36	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
76	C-8A	*Rh. sinicus*	F	195	98.36	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
77	D-17A	*Rh. sinicus*	M	195	98.91	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
78	H-80A	*Rh. sinicus*	F	196	98.37	OK017857.1	*Betacoronavirus*	*Sarbecovirus*	*unclassified Sarbecovirus*
79	C-81A	*H. armiger*	F	195	98.91	MK211378.1	*Betacoronavirus*	*Sarbecovirus*	*Coronavirus BtRs-BetaCoV/YN2018D*
80	A-109A	*Mi. schreibersii*	NA	195	96.17	KF294280.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
81	A-99A	*Mi. schreibersii*	NA	195	96.72	KF294280.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
82	H-22A	*Mi. schreibersii*	F	195	98.91	KF294280.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
83	J-56A	*Rh. sinicus*	F	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
84	J-9A	*Rh. sinicus*	F	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
85	J-110A	*Rh. sinicus*	F	195	95.63	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
86	J-120A	*Rh. sinicus*	F	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
87	J-94A	*Rh. sinicus*	F	195	95.16	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
88	J-121A	*Rh. sinicus*	M	195	94.54	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
89	J-34A	*Rh. sinicus*	M	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
90	J-58A	*Rh. sinicus*	M	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
91	J-109A	*Rh. sinicus*	M	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
92	J-36A	*Rh. sinicus*	M	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
93	B-14A	*Rh. sinicus*	F	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
94	B-16A	*Rh. sinicus*	F	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
95	B-51A	*Rh. sinicus*	M	195	95.08	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
96	H-56A	*Rh. sinicus*	F	195	80.32	KY780391.1	*unclassified Coronavirinae*	*Bat coronavirus*	*Bat coronavirus Rfer4019*
97	F-60A	*Mi. schreibersii*	F	129	96.12	KF294277.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
98	F-67A	*Mi. schreibersii*	F	100	97.03	KF294277.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
99	F-91A	*Mi. schreibersii*	M	196	89.94	KF294273.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
100	C-80A	*Rh. sinicus*	F	195	93.44	MG916902.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
101	D-37A	*H. pomona*	F	195	96.41	MZ969008.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
102	D-44A	*H. pomona*	M	195	95.9	MZ969008.1	*unclassified Coronavirinae*	*Bat coronavirus*	*NA*
103	E-37A	*My. pilosus*	M	195	100	DQ648825.1	*unclassified Coronavirinae*	*Bat coronavirus China 2005*	*Bat coronavirus (BtCoV/A604/2005)*
104	E-79A	*My. pilosus*	M	195	99.42	DQ648825.1	*unclassified Coronavirinae*	*Bat coronavirus China 2005*	*Bat coronavirus (BtCoV/A604/2005)*
105	I-6A	*Mi. schreibersii*	M	195	97.27	KU343189.1	*unclassified Coronavirinae*	*Bat coronavirus MsBtCoV/3710*	*NA*
106	I-8A	*Mi. schreibersii*	M	195	98.36	KU343189.1	*unclassified Coronavirinae*	*Bat coronavirus MsBtCoV/3710*	*NA*
107	E-57A	*Rh. affinis*	F	195	97.45	KU343198.1	*unclassified Coronavirinae*	*Bat coronavirus RaBtCoV/3750*	*NA*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
108	F-56A	*Rh. affinis*	M	195	96.43	KU343198.1	*unclassified Coronavirinae*	*Bat coronavirus RaBtCoV/3750*	*NA*
109	D-35A	*Rh. affinis*	M	195	97.96	KU343198.1	*unclassified Coronavirinae*	*Bat coronavirus RaBtCoV/3750*	*NA*
110	E-26A	*Rh. affinis*	M	195	95.41	KU343199.1	*unclassified Coronavirinae*	*Bat coronavirus RaBtCoV/4307-2*	*NA*
111	A-101A	*Rh. sinicus*	NA	195	95.41	KU343199.1	*unclassified Coronavirinae*	*Bat coronavirus RaBtCoV/4307-2*	*NA*
112	C-55A	*My. laniger*	M	195	79.08	JQ731782.1	*unclassified Coronavirinae*	*Coronavirus BtCoV/KP816/Phy_dis/PAN/2011*	*NA*
113	C-26A	*My. laniger*	M	195	79.08	JQ731782.1	*unclassified Coronavirinae*	*Coronavirus BtCoV/KP816/Phy_dis/PAN/2011*	*NA*
114	C-57A	*My. laniger*	M	195	79.08	JQ731782.1	*unclassified Coronavirinae*	*Coronavirus BtCoV/KP816/Phy_dis/PAN/2011*	*NA*
115	C-70A	*My. laniger*	M	195	79.08	JQ731782.1	*unclassified Coronavirinae*	*Coronavirus BtCoV/KP816/Phy_dis/PAN/2011*	*NA*
116	I-4A	*Me. lyra*	M	195	84.02	GU065427.1	*unclassified Coronavirinae*	*Kenya bat coronavirus BtKY83*	*NA*
117	C-29A	*My. fimbriatus*	F	195	96.11	KF569978.1	*unclassified Coronavirinae*	*Myotis daubentonii coronavirus*	*NA*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
118	C-12A	*My. fimbriatus*	F	195	97.06	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
119	C-22A	*My. fimbriatus*	F	195	96.47	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
120	C-42A	*My. fimbriatus*	F	195	99.4	KF569993.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
121	C-11A	*My. fimbriatus*	M	195	95.88	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
122	C-25A	*My. fimbriatus*	M	195	98.82	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
123	C-43A	*My. fimbriatus*	M	195	98.24	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
124	C-76A	*My. fimbriatus*	M	195	97.06	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
125	G-1A	*My. fimbriatus*	F	194	99.4	KF569993.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
126	G-7A	*My. fimbriatus*	F	195	97.06	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
127	G-2A	*My. fimbriatus*	M	195	96.47	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
128	F-22A	*My. fimbriatus*	M	196	92.98	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
129	F-27A	*My. fimbriatus*	M	195	97.6	KF569993.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
130	F-9A	*My. fimbriatus*	M	195	97.01	KF569993.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*

No.	Sample ID	Bat species	Sex	Query length (bp)	Identity %	Accession (Genbank)	Genus	Subgenus	Virus species/strain
131	H-122A	*My. fimbriatus*	M	195	97.6	KF569993.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
132	F-11A	*My. fimbriatus*	F	195	96.47	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
133	F-15A	*My. fimbriatus*	F	195	97.06	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
134	F-32A	*My. fimbriatus*	F	195	96.47	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
135	F-87A	*Rh. sinicus*	F	195	97.06	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
136	C-84A	*My. laniger*	F	164	97.4	KF569991.1	*unclassified Coronavirinae*	*Myotis davidii coronavirus*	*NA*
137	E-19A	*H. pomona*	M	96	89.58	KF569979.1	*unclassified Coronavirinae*	*Rhinolophus ferrumequinum coronavirus*	*NA*

Sample ID indicates the information of the sampling site and the bat number and the type of samples (A in the label means rectal swab). Bat sex: F (Female) and M (Male). Count of positive individuals: Alphacoronavirus (43), Betacoronavirus (36; Nobecovirus: 3, unclassified sarbecoviruses: 22 and SARSr-CoVs: 11), and unclassified Coronavirinae (58).

Table S4. Differential prevalence of coronaviruses as distributed between bat sex.

Bat Species#	Alpha/Prevalence			Beta/Prevalence			Unclassified/Prevalence			Count/ CoV prevalence			Count (All)		
	F	M	Sum	F	M	Sum	F	M	Sum	F	M	Sum	F	M	Sum
E. spelaea	0	0	0	1/100%	1/100%	2/100%	0	0	0	1/100%	1/100%	2/100%	1	1	2
H armiger	0	0	0	2/10.5%	0	2/7.4%	0	0	0	2/10.5%	0	2/7.4%	19	8	27
H. pomona	8/14.3%	8/21.1%	16/17.0%	0	0	0	1/1.8%	2/5.3%	3/3.2%	9/16.1%	10/26.3%	19/20.2%	56	38	94
Me. lyra	NA	0	0	NA	0	0	NA	1/50.0%	1/50.0%	NA	1/50%	1/50%	NA	2	2
Mi. schreibersii	3/9.7%	1/5.0%	4/7.0%	0	0	0	3/9.7%	3/15.0%	6/11.8%	6/19.4%	4/20.0%	10/19.6%	31	20	51
My. fimbriatus	1/1.9%	4/8.2%	5/4.9%	1/1.9%	0	1/1.0%	9/16.7%	9/18.4%	18/17.5%	11/20.4%	13/26.5%	24/23.3%	54	49	103
My. laniger	5/10.0%	3/7.1%	8/8.7%	0	1/2.4%	1/1.1%	1/2.0%	4/9.5%	5/5.4%	6/12.0%	8/19.0%	14/15.2%	50	42	92
My. pilosus	0	4/18.2%	4/15.4%	0	0	0	0	2/9.1%	2/7.7%	0	6/27.3%	6/23.1%	4	22	26
My. siligorensis	0	0	0	0	1/100%	1/50.0%	0	0	0	0	1/100%	1/50%	1	1	2
Rh. affinis	0	0	0	0	0	0	1/2.1%	3/11.5%	4/5.5%	1/2.1%	3/11.5%	4/5.5%	47	26	73
Rh. siamensis	0	0	0	1/6.7%	0	1/4.3%	0	0	0	1/6.7%	0	1/4.3%	15	8	23
Rh. sinicus	1/1.1%	0	1/0.7%	14/16.1%	12/19.0%	26/17.3%	10/11.5%	6/9.5%	16/10.7%	25/28.7%	18/28.6%	43/28.7%	87	63	150
Ro. amplexicaudatus	NA	0	0	NA	1/100%	1/100%	NA	0	0	NA	1/100%	1/100%	NA	1	1
Total[#]	18	20	38	19	16	35	25	30	55	62/16.5%	66/23.0%	128/19.3%	376	287	663

NA, no bats collected or no value**.** #, only the bats in which the sex was recorded are shown in this table. Alpha, the counts of individual bats with α-CoV. Beta, the counts of individual bats with β-CoV. Unclassified, the counts of individual bats with unclassified CoVs.

Table S5. Differential prevalence of α-, β-, unclassified-CoV across multiple bat genera.

S5 (a)

Genera#	**Alpha**			**Beta**			**Unclassfied**			**Count (Positive)**		
	F	M	Sum	F	M	Sum	F	M	Sum	F	M	Sum
Eonycteris	—	—	0	1	1	2	—	—	0	1	1	2
Hipposideros	8	8	16	2	—	2	1	2	3	11	10	21
Megaderma	NA	—	0	NA	—	0	NA	1	1	NA	1	1
Miniopterus	3	1	4	—	—	0	3	3	6	6	4	10
Myotis	6	11	17	1	2	3	10	15	25	17	28	45
Rhinolophus	1	—	1	15	12	27	11	9	20	27	21	48
Rousettus	NA	—	0	NA	1	1	NA	—	0	NA	1	1
Total	18	20	38	19	16	35	25	30	55	62	66	128

S5 (b)

Genera#	**Alpha-CoV prevalence**			**Beta-CoV prevalence**			**Unclassified-CoV prevalence**			**CoV prevalence**		
	F	M	Sum	F	M	Sum	F	M	Sum	F	M	Sum
Eonycteris	—	—	0.0%	100.0%	100.0%	100.0%	—	—	0.0%	100.0%	100.0%	100.0%
Hipposideros	10.7%	17.4%	13.2%	2.7%	—	1.7%	1.3%	4.3%	2.5%	14.7%	21.7%	17.4%
Megaderma	NA	—	0.0%	NA	—	0.0%	NA	50.0%	50.0%	NA	50.0%	50.0%
Miniopterus	9.7%	5.0%	7.8%	—	—	0.0%	9.7%	15.0%	11.8%	19.4%	20.0%	19.6%
Myotis	5.5%	9.6%	7.6%	0.9%	1.8%	1.3%	9.2%	13.2%	11.2%	15.6%	24.6%	20.2%
Rhinolophus	0.6%	—	0.4%	9.4%	11.7%	10.3%	6.9%	8.7%	7.6%	16.9%	20.4%	18.3%
Rousettus	NA	—	0.0%	NA	100.0%	100.0%	NA	—	0.0%	NA	100.0%	100.0%
Total	4.8%	7.0%	5.7%	5.1%	5.6%	5.3%	6.6%	10.5%	8.3%	16.5%	23.0%	19.3%

NA, no value or no bats collected. —, No prevalence of coronavirus. Alpha, the counts of individual bats with α-CoV. Beta, the counts of individual bats with β-CoV. Unclassified, the counts of individual bats with unclassified CoVs. #, only the bats in which the sex was recorded are shown in this table.

Table S6. Crosstabs analysis between coronavirus prevalence with different factors and nonparametric test.

CROSSTABS	Sampling site[a]	Bat genus[a]	Bat species[a]		Bat sex[a]
NA			Rhinolophus	Myotis	
Chi-Square Test					
Pearson χ^2	29.791***	3.473	22.648***	2.094	4.424*
Nonparametric Test: Symmetric Measure and Directional Measure					
Cramer's V	0.206***	0.070	0.296***	0.097	0.082*
Goodman-Kruskal tau	0.043***	0.005	0.088*	0.009	0.007*
N of Valid Cases	699	710	258	222	663

*, significance at p-values below the 0.05 threshold. ***, significance at p-values below the 0.001 threshold. a, 0 cells (0.0%) have expected count less than 5 in Chi-Square test.

Table S7. Differential prevalence of coronavirus in different bat species and sampling sites.

S7 (a)

Species / Sampling sites	No. of α-CoV positive samples/no. of β-CoV positive samples/no. of unclassified *Coronavirinae* positive samples#:										
	Kunming								**Xishuangbanna**	**Yuxi**	**All locations**
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	
E. spelaea	NA	NA	NA	NA	NA	NA	NA	NA	0/2/0	NA	0/2/0
H. armiger	NA	NA	0/1/0	NA	—	—	NA	0/1/0	NA	—	0/2/0
H. pomona	—	NA	1/0/0	8/0/2	7/0/1	NA	NA	NA	NA	NA	16/0/3
Me. lyra	NA	NA	NA	NA	NA	NA	NA	NA	0/0/1	NA	0/0/1
Mi. schreibersii	4/0/2	NA	NA	NA	NA	2/0/3	NA	2/0/1	0/0/2	NA	8/0/8
My. fimbriatus	NA	1/0/0	1/0/8	NA	NA	0/0/6	0/0/3	3/1/1	NA	NA	5/1/18
My. laniger	NA	—	8/1/5	NA	NA	—	—	—	NA	—	8/1/5
My. pilosus	NA	NA	NA	NA	4/0/2	—	NA	—	NA	NA	4/0/2
My. siligorensis	NA	—	NA	NA	NA	NA	NA	NA	NA	0/1/0	0/1/0
Rh. affinis	—	NA	—	0/0/1	0/0/2	0/0/1	NA	NA	NA	—	0/0/4
Rh. siamensis	NA	NA	—	NA	NA	NA	NA	0/1/0	NA	—	0/1/0
Rh. sinicus	1/1/1	0/0/3	0/3/1	0/2/0	NA	0/2/1	NA	1/8/1	—	0/11/10	2/27/17
Ro. amplexicaudatus	NA	NA	NA	NA	NA	NA	NA	NA	0/1/0	NA	0/1/0
All species	5/1/3	1/0/3	10/5/14	8/2/3	11/0/5	2/2/11	0/0/3	6/11/3	0/3/3	0/12/10	43/36/58

. #NA, no bats collected. #—, no prevalence of coronavirus

S7 (b)

Sampling sites **Species**	**Prevalence of α-CoV/β-CoV/unclassified *Coronavirinae*#:**										
	Kunming								**Xishuangbanna**	**Yuxi**	**All locations**
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	
E. spelaea	NA	NA	NA	NA	NA	NA	NA	NA	0.0/100.0/0.0%	NA	0.0/100.0/0.0%
H. armiger	NA	NA	0.0/20.0/0.0%	NA	—	—	NA	0.0/25.0/0.0%	NA	—	0.0/7.4/0.0%
H. pomona	—	NA	100.0/0.0/0.0%	18.2/0.0/4.5%	14.6/0.0/2.1%	NA	NA	NA	NA	NA	16.7/0.0/3.1%
Me. lyra	NA	NA	NA	NA	NA	NA	NA	NA	0.0/0.0/50.0%	NA	0.0/0.0/50.0%
Mi. schreibersii	57.1/0.0/28.6%	NA	NA	NA	NA	5.9/0.0/8.8%	NA	33.3/0.0/16.7%	0.0/0.0/66.7%	NA	13.3/0.0/13.3%
My. fimbriatus	NA	11.1/0.0/0.0%	5.0/0.0/40.0%	NA	NA	0.0/0.0/28.6%	0.0/0.0/60.0%	6.1/2.0/2.0%	NA	NA	4.8/1.0/17.3%
My. laniger	NA	—	17.4/2.2/10.9%	NA	NA	—	—	—	NA	—	8.7/1.1/5.4%
My. pilosus	NA	NA	NA	NA	21.1/0.0/10.5%	—	NA	—	NA	NA	15.4/0.0/7.7%
My. siligorensis	NA	—	NA	NA	NA	NA	NA	NA	NA	0.0/100.0/0.0%	0.0/50.0/0.0%
Rh. affinis	—	NA	—	0.0/0.0/50.0%	0.0/0.0/8.7%	0.0/0.0/3.4%	NA	NA	NA	—	0.0/0.0/4.2%
Rh. siamensis	NA	NA	—	NA	NA	NA	NA	0.0/12.5/0.0%	NA	—	0.0/4.0/0.0%
Rh. sinicus	9.1/9.1/9.1%	0.0/0.0/13.6%	0.0/37.5/12.5%	0.0/100.0/0.0%	NA	0.0/8.0/4.0%	NA	1.5/12.1/1.5%	—	0.0/40.7/37.0%	1.2/16.7/10.5%
Ro. amplexicaudatus	NA	NA	NA	NA	NA	NA	NA	NA	0.0/100.0/0.0%	NA	0.0/100.0/0.0%
All species	11.1/2.2/6.7%	1.4/0.0/4.3%	11.2/5.6/15.7%	16.7/4.2/6.3%	10.8/0.0/4.9%	1.6/1.6/8.9%	0.0/0.0/37.5%	4.4/8.1/2.2%	0.0/33.3/33.3%	0.0/14.0/11.6%	6.0/5.0/8.1%
Total	20.0%	5.8%	32.6%	27.1%	15.7%	12.1%	37.5%	14.7%	66.7%	25.6%	19.1%

NA, no value or no bats collected. —, No prevalence of coronavirus.

Table S8. Differential prevalence of CoV co-existing in individual bat samples.

No.	Sample label	DSI %	BLAST TaxID	Genus	Virus species
1	C-29A-1	82.6	1906673	*Alphacoronavirus*	*Alphacoronavirus sp.*
	C-29A-2	13.9	1244203	*Alphacoronavirus*	*Bat coronavirus HKU10*
	C-29A-3	1.8	1508220	*unclassified Coronavirinae*	*Bat coronavirus*
2	C-42A-1	88.2	1487702	*unclassified Coronavirinae*	*Myotis davidii coronavirus*
	C-42A-2	7.7	1487702	*unclassified Coronavirinae*	*Myotis davidii coronavirus*
	C-42A-3	3.3	1487701	*unclassified Coronavirinae*	*Myotis daubentonii coronavirus*
3	C-86A-1	84.2	1906673	*Alphacoronavirus*	*Alphacoronavirus sp.*
	C-86A-2	10.3	393045	*Alphacoronavirus*	*Bat coronavirus HKU6*
	C-86A-3	2.2	1487701	*unclassified Coronavirinae*	*Myotis daubentonii coronavirus*
	C-86A-4	1.7	1487701	*unclassified Coronavirinae*	*Myotis daubentonii coronavirus*

DSI: Diversity of sequences in individual bats.

Table S9. Crosstabs analysis between differential prevalence of CoV with different factors and nonparametric test.

CROSSTABS	Sampling site[a]	Bat genus[b]	Bat species[c]	Bat sex[b]	Location[d]
Chi-Square Test					
Pearson χ^2	46.564***	56.203***	81.907***	0.693	21.222***
Nonparametric Test: Symmetric Measure and Directional Measure					
Cramer's V	0.450***	0.490***	0.572***	0.074	0.278***
Goodman-Kruskal tau	0.197***	0.223***	0.303	0.003	0.069***
N of Valid Cases	115	117	125	128	137

*, significance at p-values below the 0.05 threshold. ***, significance at p-values below the 0.001 threshold. a, 5 cells (27.8%), b, 0 cells (0.0%), c, 7 cells (38.9%), d, 3 cells (33.3%) have expected count less than 5 in Chi-Square test.

Table S10. Host verification and virus species of 11 SARSr-CoV positive samples.

No.	Sample ID[a]	Query length (bp)	Query cover	Percent identity	Accession (Genbank)	Bat species (COI gene)	Subgenus	Virus species
1	A-92A	195	93%	98.91	KY417149.1	*Rh. cf. thomasi*	*Sarbecovirus*	*Bat SARS-like coronavirus Rs4255*
2	C-40A	195	93%	99.45	FJ588686.1	*Rh. cf. thomasi*	*Sarbecovirus*	*SARS coronavirus Rs_672/2006*
3	C-45A	195	94%	99.46	KY417145.1	*My. laniger*	*Sarbecovirus*	*Bat SARS-like coronavirus Rf4092*
4	D-16A	156	91%	100	KY417145.1	*Rh. cf. thomasi*	*Sarbecovirus*	*Bat SARS-like coronavirus Rf4092*
5	F-92A	195	93%	98.91	FJ588686.1	*Rh. cf. thomasi*	*Sarbecovirus*	*SARS coronavirus Rs_672/2006*
6	H-15A	195	93%	96.34	KY417149.1	*Rh. cf. thomasi*	*Sarbecovirus*	*Bat SARS-like coronavirus Rs4255*
7	H-54A	195	93%	98.91	KY417149.1	*Rh. cf. thomasi*	*Sarbecovirus*	*Bat SARS-like coronavirus Rs4255*
8	H-72A	195	93%	98.91	KY417149.1	*Rh. cf. thomasi*	*Sarbecovirus*	*Bat SARS-like coronavirus Rs4255*
9	H-75A	195	93%	98.91	KY417149.1	*Rh. siamensis*	*Sarbecovirus*	*Bat SARS-like coronavirus Rs4255*
10	H-82A	195	93%	98.91	KY417149.1	*Rh. cf. thomasi*	*Sarbecovirus*	*Bat SARS-like coronavirus Rs4255*
11	H-135A	195	93%	98.91	KY417149.1	*Rh. cf. thomasi*	*Sarbecovirus*	*Bat SARS-like coronavirus Rs4255*

a, Sample ID indicates the information of the sampling site and the bat number and the type of samples (A in the label means rectal swab).

Table S11. Statistics for contigs mapping to whole genome and different genes of SARSr-CoVs.

Gene	Whole		ORF1a		ORF1b		S		3a		E	
Size*	29743 bp		13149 bp		7887 bp		3726 bp		825 bp		231 bp	
Mapping	Coverage	Identity	Coverage	Identity	Coverage	Identity	Coverage	Identity	Coverage	Identity	Coverage	Identity
Pool 41	18369	93.9	6543	94.2	4752	93.2	3152	93.2	741	96.0	NA	NA
Pool 96	20241	91.0	8773	91.2	5464	91.5	2447	90.3	758	94.5	168	33.1
Pool 100	208	87.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pool 111	23539	89.6	8938	91.5	6135	93.9	3726^	72.7	784	86.6	231^	98.3
Pool 114	29567^	91.7	12973	97.8	7887^	96.3	3726^	76.7	825^	89.9	231^	98.7
Pool 115	24095	92.6	10398	94.6	6297	93.5	2832	90.0	825^	87.7	231^	98.7
Pool 117	10565	92.0	3839	92.2	1776	86.7	1573	94.5	444	85.4	NA	NA
Size#	29059 bp		12570 bp		7887 bp		3726 bp		825 bp		231 bp	
Pool 99	2189	90.6	530	91.5	952	90.4	NA	NA	NA	NA	NA	NA
Pool 109	2216	86.1	777	92.3	295	88.3	NA	NA	NA	NA	NA	NA

Gene	M		6		7a		7b		8		N	
Size*	693 bp		192 bp		369 bp		135 bp		366 bp		1269 bp	
Mapping	Coverage	Identity	Coverage	Identity	Coverage	Identity	Coverage	Identity	Coverage	Identity	Coverage	Identity
Pool 41	557	95.3	192^	97.6	208	90.4	135^	98.5	366^	89.2	1269^	95.4
Pool 96	693^	92.8	192^	98.4	123	84.6	NA	NA	200	90.0	1149	95.0
Pool 100	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	208	87.0
Pool 111	693^	95.0	192^	99.5	369^	90.3	135^	99.3	366^	97.8	1269^	97.9
Pool 114	693^	98.1	192^	99.5	369^	98.5	135^	98.5	366^	98.9	1269^	97.9
Pool 115	690	79.2	192^	38.1	369^	97.6	135^	98.5	366^	98.1	1269^	98.2
Pool 117	559	95.3	192^	97.4	25	32.0	123	87.8	366^	97.8	1265^	93.7
Size#	693 bp		192 bp		369 bp		135 bp		366 bp		1269 bp	
Pool 99	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	707	90.3
Pool 109	NA	NA	NA	NA	NA	NA	NA	NA	226	39.0	904	91.6

*, size of the reference sequence (GenBank: KY417149.1); #, size of the reference sequence (GenBank: FJ588686.1); ^, the coverage for the size of references is more than 99%. NA, no coverage.

Table S12. The information of the CoV-positive bats with ectoparasite.

No.	Bat ID[a]	Bat species	CoVs positive
1	B-14	*Rh. sinicus*	*unclassified Coronavirinae*
2	C-22	*My. fimbriatus*	*unclassified Coronavirinae*
3	C-55	*My. laniger*	*unclassified Coronavirinae*
4	E-37	*My. pilosus*	*unclassified Coronavirinae*
5	E-75	*My. pilosus*	*Alphacoronavirus*
6	E-76	*My. pilosus*	*Alphacoronavirus*
7	E-79	*My. pilosus*	*unclassified Coronavirinae*
8	E-84	*My. pilosus*	*Alphacoronavirus*
9	F-9	*My. fimbriatus*	*unclassified Coronavirinae*
10	F-32	*My. fimbriatus*	*unclassified Coronavirinae*
11	F-56	*Rh. affinis*	*unclassified Coronavirinae*
12	F-58	*Mi. schreibersii*	*Alphacoronavirus*
13	F-59	*Mi. schreibersii*	*Alphacoronavirus*
14	F-87	*Rh. sinicus*	*unclassified Coronavirinae*
15	H-22	*Mi. schreibersii*	*unclassified Coronavirinae*
16	H-96	*Mi. schreibersii*	*Alphacoronavirus*
17	I-1	*Ro. amplexicaudatus*	*Betacoronavirus*
18	I-3	*E. spelaea*	*Betacoronavirus*
19	I-4	*Me. lyra*	*unclassified Coronavirinae*
20	I-6	*Mi. schreibersii*	*unclassified Coronavirinae*
21	I-8	*Mi. schreibersii*	*unclassified Coronavirinae*

a, Bat ID indicates the information of the sampling site and the bat number.

Table S13. Multinominal Logistic regression (Main effects) analysis used to depict risk factors in CoV prevalence and differential distribution among bats

Multifactor models (Main effects)	**Likelihood Ratio Tests**		**Model Fitting Criteria**		**Pseudo R^2**	
	Factors	P value	AIC[c]	BIC[d]	Nagelkerke	McFadden
CoV Prevalence[a] (Dependent)	Site+Sex+Species	0.000	213.735	321.657	0.244	0.169
	Site^	0.000	256.875	328.823	NA	NA
	Sex^	0.372	212.530	315.956	NA	NA
	Species^	0.000	251.110	296.078	NA	NA
	Site+Sex+Genus	0.000	171.288	243.236	0.118	0.078
	Site	0.000	191.090	227.064	NA	NA
	Sex	0.161	171.249	238.701	NA	NA
	Genus	0.404	165.461	210.429	NA	NA
CoV Differential Distribution[b] (Dependent)	Site+Sex+Species	0.000	142.879	268.368	0.765	0.524#
	Site^	0.038	138.189	218.046	NA	NA
	Sex^	0.492	140.295	260.081	NA	NA
	Species^	0.000	171.348	228.388	NA	NA
	Site+Sex+Genus	0.000	132.931	224.196	0.678	0.424#
	Site^	0.002	137.631	183.263	NA	NA
	Sex^	0.842	129.274	214.835	NA	NA
	Genus^	0.000	157.881	214.921	NA	NA

NA, no value; a, category in CoV positive and negative, 663 bat individuals; b, category in α-CoV, β-CoV and unclassified CoV positive, 128 bat individuals; c, AIC: Akaike information criterion; d, Bayesian information criterion; +, no consideration of interaction effect in different variables; ^, AIC and BIC are of reduced model in Likelihood Ratio Tests. #, MacFadden value >0.2.

Table S14. Multinominal Logistic regression analysis (Full factorial) used to depict risk factors in CoV prevalence and differential distribution among bats

Multifactor models (Full factorial)	**Likelihood Ratio Tests**		**Model Fitting Criteria**		**Pseudo R^2**	
	Factors	P value	AIC	BIC	Nagelkerke	McFadden
CoV Prevalence[a] (Dependent)	Site * Sex * Species	0.000	265.651	661.367	0.391	0.286#
	Site * Sex^	NA	265.651	661.367	NA	NA
	Site * Species^	NA	265.651	661.367	NA	NA
	Sex * Species^	NA	265.651	661.367	NA	NA
	Site * Sex * Species^	0.999	237.771	561.539	NA	NA
	Site * Sex * Genus	0.000	191.472	425.304	0.229	0.158
	Site * Sex^	NA	191.472	425.304		
	Site * Genus^	NA	191.472	425.304		
	Sex * Genus^	NA	191.472	425.304		
	Site * Sex * Genus^	0.505	179.755	364.123		
CoV Differential Distribution[b] (Dependent)	Site * Sex * Species	0.000	210.259	461.238	0.820	0.598#
	Site * Species^	NA	210.259	461.238	NA	NA
	Site * Sex^	NA	210.259	461.238	NA	NA
	Species * Sex^	NA	210.259	461.238	NA	NA
	Site * Species * Sex^	1.000	202.259	441.830	NA	NA
	Site * Sex * Genus	0.000	192.854	415.313	0.778	0.540#
	Site * Sex^	NA	192.854	415.313	NA	NA
	Site * Genus^	NA	192.854	415.313	NA	NA
	Sex * Genus^	NA	192.854	415.313	NA	NA
	Site * Sex * Genus^	1.000	180.855	386.201	NA	NA

NA, no value; a, category in CoV positive and negative, 663 bat individuals; b, category in α-CoV, β-CoV and unclassified CoV positive, 128 bat individuals; c, AIC: Akaike information criterion; d, Bayesian information criterion; *, statistical interaction in different variables; ^, AIC and BIC are of reduced model in Likelihood Ratio Tests. #, MacFadden value >0.2.

Table S15. Deposition of high-throughput sequencing data in open access database.

Sample accession	Sample ID	Counterpart bat individual	Accession (NMDC*)
Illumina sequencing			
NMDC20070104	YN-pool 41	H-15A	NMDC40041234
NMDC20070105	YN-pool 51	C-45A	NMDC40041235
NMDC20070106	YN-pool 96	H-75A	NMDC40041236
NMDC20070107	YN-pool 99	C-40A	NMDC40041237
NMDC20070108	YN-pool 100	D-16A	NMDC40041238
NMDC20070109	YN-pool 109	F-92A	NMDC40041239
NMDC20070110	YN-pool 111	A-92A	NMDC40041240
NMDC20070111	YN-pool 114	H-54A	NMDC40041241
NMDC20070112	YN-pool 115	H-82A and H-135A	NMDC40041242
NMDC20070113	YN-pool 117	H-72A	NMDC40041243
MGI sequencing			
NMDC20070114	YN-P32-22	C-22A	NMDC40041244
NMDC20070115	YN-P32-86	C-86A	NMDC40041245
NMDC20070116	YN-P32-12	C-12A	NMDC40041246
NMDC20070117	YN-P32-29	C-29A	NMDC40041247
NMDC20070118	YN-P32-42	C-42A	NMDC40041248
NMDC20070119	YN-P33-25	C-25A	NMDC40041249
NMDC20070120	YN-P33-76	C-76A	NMDC40041250
NMDC20070121	YN-P33-11	C-11A	NMDC40041251
NMDC20070122	YN-P33-43	C-43A	NMDC40041252

* NMDC, China National Microbiology Data Center (https://nmdc.cn/en)

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