

# Coevolution of Coronavirus and Paramyxovirus with Their Bat Hosts in the Same Geographical Areas

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## Research article

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## Abstract

Bat-borne viruses are relatively host specific. In this study, coevolution analyses were conducted on coronaviruses and paramyxoviruses and their bat hosts to investigate the potential mechanisms of such host specificity. The published nucleotide sequences of the RNA dependent RNA polymerase (RdRp) gene of 60 coronaviruses from 37 bat species and those of the RNA polymerase large (L) gene of 36 paramyxoviruses from 29 bat species were analyzed. Each of the coevolution signal detected was tested and verified by the ParaFit and PACo functions of the R program. Significant coevolution signals were detected in coronaviruses and paramyxoviruses and their bat hosts, and closely related bat hosts were found to carry viruses that are closely related. Results also suggest that similar geographical distribution and close phylogenetic relationship may have resulted in infection of different bat species by the same strain of virus. As the natural hosts of certain viruses were mostly found in their endemic and surrounding areas, we speculate that the ancestors of bat hosts of Middle East respiratory syndrome coronaviruses (MERS-CoVs) may belong to the family of Vespertilionidae and are evolutionarily close to *Neoromicia capensis* and *Pipistrellus hesperidus* bats that are present in Africa and west Asia. In addition, we speculate that bat coronaviruses that are closely related to the novel coronavirus 2019 (COVID-19) may be found in bats related to *Rhinolophus affinis*. Although the coevolution between viruses and bat host is not surprised, this is the first systematical summary elucidating the relationship between coronaviruses, paramyxoviruses, host and geographical areas. It provides a theoretical basis for the viruses trace.

## Significance

Bat-borne coronaviruses and paramyxoviruses have caused a number of outbreaks worldwide, posing a great threat to the safety of human life and property. It is important to understand the bats-viruses evolutionary history and rules for the virus tracing. In our long-term surveillance of bat viruses, we found that they are family, genus or species-specific. In this study, we are committed to show this phenomena through co-phylogenetic analysis. Host distribution were taken into account as an important analytical factor in this study. Our results suggest that similar hosts carry similar viruses, similar distribution area may facilitate inter-species transmission of bat viruses, and several endemic area were overlap with the natural host distribution. This study is of great significance for further study of bats-viruses evolutionary history and enhanced our awareness of virus prevention and control.

# J. Liang and C. Zhu contributed equally to this study.

## Introduction

Bats are reservoirs of many zoonotic viruses, such as members of *Filoviridae* (e.g., Ebola and Marburg viruses), *Paramyxoviridae* (e.g., Hendra and Nipah viruses), and *Coronaviridae* (e.g., severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus, and novel coronavirus-2019) (Luis et al. 2013; Zhou and Yang 2020). Bats live in a wide variety of environments with various feeding habits. They are flying mammals and are effective vehicles for spreading viruses (Serra-Cobo and López-Roig 2016). Bats have been shown to be responsible for the global outbreaks of severe acute respiratory syndrome (SARS) in 2002–2003, Middle East respiratory syndrome (MERS) in 2012 (Zaki et al. 2012, Drexler et al. 2014) and COVID-19 recently (Zhou and Yang 2020).

Hendra virus (HeV) and Nipah virus (NiV) are highly pathogenic zoonoses and have been detected in flying-fox bats (*Pteropus spp.*) (Smith et al. 2011; Anderson et al. 2019). HeV was first identified in Queensland, Australia in 1994, causing acute respiratory disease and febrile illness in horses and humans who have close contact with sick horses (Selvey et al. 1995). Since 1994, 7 human cases of HeV infection were reported, and four of them were fatal (<https://www.who.int/emergencies/diseases/hendra-virus/en/>; <https://www.cdc.gov/vhf/hendra/index.html>).

NiV was first detected in Malaysia in 1999 during the outbreak of encephalitis and respiratory illness among pig farmers. Approximately 300 human cases of NiV infection with over 100 deaths have been reported, and sporadic outbreaks have occurred in Malaysia, Singapore, Bangladesh, the Philippines, and India (<https://www.who.int/news-room/fact-sheets/detail/nipah-virus>; <https://www.cdc.gov/vhf/nipah/index.html>).

Host-parasite specificity has been shown in malaria parasites (Ricklefs et al. 2004), bat flies (Nikon et al. 2011), bacteria (Lei and Olival 2014), and coronaviruses (Cui et al. 2007). As an ancient mammalian species (Teeling et al. 2005), bats may have coevolved with their parasites. In this study, we use the bat *cyt b* gene and viral replicase polymerase sequence as the analysis object, for they are one of the most conservative fragment in the mammalian and viral genome, and more suitable for elucidating evolutionary relationships. Secondly, due to the stability of replicase polymerase makes them more readily available. Such as HKU6 and HKU7, we can get RNA dependent RNA polymerase (RdRp) of them but Spike is unavailable. We had also analyzed 440 bp and 816 bp coronavirus replicase fragments of hundreds of coronavirus sequence, but confidence interval of the evolutionary tree is too low. For getting more sequences of diverse viruses that long enough and from various states, we analyzed the complete nucleotide sequences of the RdRp gene of 60 coronaviruses from 37 bat species and 559 bp partial sequences of the RNA polymerase large (L) gene of 36 paramyxoviruses from 29 bat species to examine their phylogenetic patterns.

## Methods

Bat Collection and Gene Amplification

The cytochrome b (*cyt b*) sequences of *Tylonycteris robustula* (MN366287, Family: *Vespertilionidae*) and *Miniopterus pusillus* (MN366288, Family: *Miniopteridae*) were used for host-pathogen coevolution analyses. Anal swabs and patagiums of the two species were collected from Menghai, Yunnan and Kau O Bat Cave, Macau, respectively. Bats were released in their roosts after sample collection.

The DNA fragment containing the *cyt b* gene was amplified with primers L14727ag (5'- ATGATATGAAAAACCATCGTTG) and H15915ag (5'- TTTCNTTCTGGTTTACAAGAC) (Guillén-Servent and Francis 2006) under the following conditions: 94 °C for 3 min, followed by 20 cycles of 94 °C for 20 s, 46 °C to 52 °C (+ 0.3 °C/cycle) for 30 s, and 72 °C for 90 s and 30 cycles of 94 °C for 20 s, 60 °C for 30 s, 72 °C for 90 s and then maintained at 72 °C for 10 min. The PCR products were sequenced. The sequences thus obtained have been deposited in the Genbank with accession numbers MN366287 and MN366288.

## Phylogenetic Analysis

The entire nucleotide sequences of 60 coronaviruses and 37 paramyxoviruses including outgroups were downloaded from the Database of Bat-associated Viruses (DBatVir, <http://www.mgc.ac.cn/DBatVir/>) in the Genbank. Sequences were aligned with Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo>). The outgroups included in each alignment were Turkey COV/NC\_01080 for coronavirus, Sosuga virus/KF774436 for paramyxovirus, and Megaderma lyra/DQ888678 for bats. Maximum-likelihood phylogenetic trees of viruses and their hosts were constructed using 1000 bootstraps with the raxmlGUI program (Silvestro and Michalak 2012). The GTR + I + G of the nucleotide substitution model was chosen for analysis with the jmodeltest 2.1.7 software (Darriba et al. 2012).

## Global-fit Analysis

The degree of congruence of phylogenetic topologies between bats and viruses were identified using the global-fit method of ParaFit (Legendre et al. 2002). The matrices of patristic distances were calculated from the maximum likelihood tree of host and virus phylogenies using the “cophenetic” function of the package ape (Paradis et al. 2004) in R 3.6.0 (R Core Team 2013). ParaFit analyses were performed with 999 permutations in global and individual link tests. Each individual host-virus interaction was considered as significant when its ParaFit 1 or Parafit 2 P-value was  $\leq 0.05$  (Lei and Olival 2014). To verify the results, each phylogenetic signal was tested using the Procrustean Approach to Cophylogeny (PACo) (Balbuena et al. 2013), which differs from ParaFit in that the virus matrix is rotated and scaled to fit the host matrix. A goodness-of-fit test based on 1000 randomizations was used to assess significance. The associated squared residuals were used to assess the importance of each host–virus link (Singh et al. 2016). To visualize bat-virus associations, their cophylogenetic trees were generated using the “cophylo” function of the R package phytools (Revell 2011).

## Results

**Bat-coronavirus.** Results of both ParaFit and PACo analyses of RdRp gene sequences showed evidence of coevolution between coronaviruses (CoVs) and their bat hosts (ParaFitGlobal = 390.8896,  $P = 0.001$ ;  $m^2$  global value = 57.136,  $P \leq 0.001$ ). Fifty-one of the 60 individual host-parasite links were significant with a ParaFit1 or ParaFit2 P value  $\leq 0.05$ .

MERS related coronaviruses (MERSr-CoVs) were detected in bats from Europe, Africa, and Asia. All of these bats belonged to the family Vespertilionidae (Fig. 1). Bat-derived MERSr-CoV isolates designated PML – PHE1/RSA/KC869678, 5038/RSA/MF593268, and PDF – 2180/UG/KX574227 had the highest (> 91%) nucleotide sequence identity with human and camel MERS-CoVs (Table 1). Isolates PML – PHE1/RSA/KC869678 and 5038/RSA/MF593268 were detected in *Neoromicia capensis* bats, members of the *Vespertilionidae* family, from South Africa. Isolate PDF – 2180/UG/KX574227 was detected in *Pipistrellus hesperidus* bats, which are also members of the *Vespertilionidae* family, from Uganda (Fig. 1). Isolate PDF – 2180/UG/KX574227 and its closest MERS-CoVs from humans and camels shared nearly 92% nucleotide sequence identity (Table 1). It also shared 91.93% nucleotide sequence identity with isolate 5038/RSA/MF593268 and 91.79% with isolate PML – PHE1/RSA/KC869678. Both isolates 5038/RSA/MF593268 and PML – PHE1/RSA/KC869678 shared > 93% nucleotide sequence identity with their closest MERS-CoVs from camels and humans (Table 1). Other MERSr-CoV isolates, including HKU5 – 1/CN/EF065509, HKU4 – 1/CN/EF065505, GX2012/CN/KJ473822, JPDB144/CN/KU182965, and GD2013/CN/KJ473820 shared < 83% nucleotide sequence identity with MERS-CoVs from camels and humans (Table 1). MERSr-CoV isolates PML – PHE1/RSA/KC869678, 5038/RSA/MF593268, NL13845/CN/MG021451, HKU25/CN/KX442565, SC2013/CN/KJ473821 206645 – 40/IT/MG596802, and 206645 – 63/IT/MG596803 shared > 85% nucleotide sequence identity with their closest camel and human MERS-CoVs (Table 1). As shown in Fig. 1, the host bats of isolates HKU5 – 1/CN/EF065509, HKU4 – 1/CN/EF065505, GX2012/CN/KJ473822, JPDB144/CN/KU182965, and GD2013/CN/KJ473820 are distantly related to *N. capensis* bats (Fig. 1). Isolates 206645 – 40/IT/MG596802 and 206645 – 63/IT/MG596803 detected in *Hypsugo savii* and *P. kuhli* bats, respectively (Fig. 1), from Italy shared 99.46% nucleotide sequence identity with each other.

Table 1  
RdRp nucleotide sequence identity between MERSr-CoVs and camel and human MERS-CoVs.

Bat MERSr- CoVs	Host	Closest camel MERS-CoVs		Closest human MERS-CoVs		Close bat species*
		CoV name	identity	CoV name	identity	
5038/RSA/MF593268	<i>Neoromicia capensis</i>	B78/UAE/MG598668	93.77%	2285/OMA/KT156560	93.77%	<i>Neo cap</i>
PML-PHE1/RSA/KC869678	<i>Neoromicia capensis</i>	T157/KSA/KT368890	93.49%	C20860/KSA/KT806055	93.49%	<i>Neo cap</i>
PDF-2180/UG/KX574227	<i>Pipistrellus hesperidus</i>	B25/UAE/MF598617	91.99%	016/KSA/MN120513	91.95%	<i>Pip hes</i>
206645-63/CN/MG596803	<i>Pipistrellus kuhlii</i>	047(b)/KSA/KT368852	86.20%	016/KSA/MN120513	86.13%	<i>Pip hes</i>
NL13845/CN/MG021451	<i>la io</i>	415915/UAE/MF598718	86.16%	11958/KSA/KX154687	86.20%	<i>Neo cap</i>
206645-40/IT/MG596802	<i>Hypsugo savii</i>	047(b)/KSA/KTKT368852	86.10%	016/KSA/MN120513	86.03%	<i>Neo cap</i>
HKU25/CN/KX442565	<i>Hypsugo pulveratus</i>	HKU44/ETH/MG923468	85.80%	10717/KSA/MH310911	85.72%	<i>Neo cap</i>
SC2013/CN/KJ473821	<i>Vespertilio sinensis</i>	415915/UAE/MF598718	85.72%	Bisha/KSA/KF600620	85.72%	<i>Neo cap</i>
GX2012/CN/KJ473822	<i>Tylonycteris pachypus</i>	HKU213/MON/MF598718	81.70%	2362/KSA/MH371127	81.92%	<i>Pip hes</i>
HKU5-1/CN/EF065509	<i>Pipistrellus abramus</i>	T157/KSA/KT368890	82.99%	016/KSA/MN120513	83.06%	<i>Pip hes</i>
GD2013/CN/KJ473820	<i>Pipistrellus abramus</i>	T157/KSA/KT368890	82.88%	18012493/KSA/MK462249	82.92%	<i>Pip hes</i>
JPDB144/CN/KU182965	<i>Myotis daubentonii</i>	047(b)/KSA/KTKT368852	81.93%	20570/KSA/MG011354	81.97%	<i>Pip hes</i>
HKU4-1/CN/EF065505	<i>Tylonycteris pachypus</i>	047(b)/KSA/KTKT368852	81.81%	20570/KSA/MG011354	81.84%	<i>Pip hes</i>

\*Neo cap = *Neoromicia capensis*; Pip hes = *Pipistrellus hesperidus*

*Rhinolophus sinicus* and *R. ferrumequinum* bats were most commonly found to harbor SARS related coronaviruses (SARSr-CoVs) (Fig. 1). *R. sinicus* bats are distributed in Asia, and *R. ferrumequinum* are present in Africa, Asia, and Europe (Fig. 1). Rs3367/CN/KC881006 and YN2013/CN/KJ473816 shared the highest nucleotide identity with SARS-CoV (Table 2) they were detected from *R. sinicus*. The SARSr-CoV isolate designated 16B0133/Korea/KY938558 was detected in *R. ferrumequinum* from South Korea. This isolate was found to be evolutionarily close to SARSr-CoV isolates JL2012/KJ473811 and JTMC15/KU182964 found in the same host species, *R. ferrumequinum*, from Jilin, China (Fig. 1), which is located north of South Korea. Isolate 16B0133/Korea/KY938558 shared 99.71% nucleotide sequence identity with isolate JL2012/KJ473811 and 99.68% with isolate JTMC15/KU182964, JL2012/CN/KJ473811 and JTMC15/CN/KU182964 shared 99.96% to each other (Table 2).

Table 2

RdRp nucleotide sequence identity between SARSr-CoVs from various species of bats and their closest bat CoVs and human SARS-CoVs.

Bat SARSr- CoVs	Host	Closest bat CoVs		Closest human SARS-CoVs	
		CoV name	Identity	CoV name	identity
JL2012/CN/KJ473811	<i>Rhinolophus ferrumequinum</i>	JTMC15/CN/KU182964	99.96%	Sin847/SIN/AY559095	92.42%
JTMC15/CN/KU182964	<i>Rhinolophus ferrumequinum</i>	JL2012/CN/KJ473811	99.96%	Sin847/SIN/AY559095	92.38%
16B0133/ROK/KY938558	<i>Rhinolophus ferrumequinum</i>	JL2012/CN/KJ473811	99.71%	Sin847/SIN/AY559095	92.42%
Rf1/CN/DQ412042	<i>Rhinolophus ferrumequinum</i>	273/CN/DQ648856	99.64%	icSARS-C7-MA/USA/MK062184	92.45%
SX2013/CN/KJ473813	<i>Rhinolophus ferrumequinum</i>	HeB2013/CN/KJ473812	99.71%	icSARS-C7-MA/USA/MK062184	92.95%
HeB2013/CN/KJ473812	<i>Rhinolophus ferrumequinum</i>	SX2013/CN/KJ473813	99.71%	icSARS-C7-MA/USA/MK062184	93.13%
Shaanxi2011/CN/JX993987	<i>Rhinolophus pusillus</i>	HeB2013/CN/KJ473812	95.60%	icSARS-C7-MA/USA/MK062184	93.13%
HKU3 - 1/CN/DQ022305	<i>Rhinolophus sinicus</i>	HKU3 - 1/CN/DQ084200	100.00%	Sin846/SIN/AY559094	91.73%
HuB2013/CN/KJ473814	<i>Rhinolophus sinicus</i>	Rm1/CN/DQ412043	95.13%	HZS2-E/CN/AY394990	93.24%
Rm1/CN/DQ412043	<i>Rhinolophus macrotis</i>	279/CN/DQ648857	99.79%	JMD/CN/AY394988	93.56%
Yunnan2011/CN/JX993988	<i>Chaerephon plicatus</i>	MLHJC35/CN/KU182963	97.03%	Sin847/SIN/AY559095	94.96%
Anlong - 103/CN/KY770858	<i>Rhinolophus sinicus</i>	Anlong - 112/CN/KY770859	100.00%	JMD/CN/AY394988	98.39%
GX2013/CN/KJ473815	<i>Rhinolophus sinicus</i>	Anlong - 112/CN/KY770859	98.35%	HZS2-E/CN/AY394990	97.64%
Rs3367/CN/KC881006	<i>Rhinolophus sinicus</i>	WIV1/CN/KF367457	99.93%	JMD/CN/AY394988	98.39%
YN2013/CN/KJ473816	<i>Rhinolophus sinicus</i>	YN2018B/CN/MK211376	99.57%	JMD/CN/AY394988	98.39%
As6526/CN/KY417142	<i>Aselliscus stoliczkanus</i>	YN2018B/CN/MK211376	99.89%	JMD/CN/AY394988	98.28%
Rp3/CN/DQ071615	<i>Rhinolophus pearsonii</i>	Anlong-29/CN/KF294439	99.03%	JMD/CN/AY394988	97.96%
LYRa11/CN/KF569996	<i>Rhinolophus affinis</i>	Rf4092/CN/KY417145	98.06%	JMD/CN/AY394988	96.89%
BM48 - 31/BGR/GU190215	<i>Rhinolophus blasii</i>	Rs4231/CN/KY417146	88.42%	HZS2-E/CN/AY394990	88.18%
RaTG13/CN/MN996532	<i>Rhinolophus affinis</i>	SNU01/ROK/MT039890	97.85%	SZ16/HK/AY304488	88.26%

SARSr-CoVs were also detected in bats outside the *Rhinolophidae* family. The isolate As6526/CN/KY417142 from *Aselliscus stoliczkanus* bats (family: *Hipposideridae*), which are evolutionarily close to *Rhinolophidae* bats, shared 99.89% nucleotide sequence identity with isolates YN2018B/CN/MK211376 found in *R. affinis* bats from Yunnan, China (Table 2). Isolate Yunnan2011/CN/JX993988 found in *Chaerephon plicatus* bats, which are relatively distant from *Rhinolophus* bats (Fig. 1), also shared 97.03% nucleotide sequence identity with isolate MLHJC35/CN/KU182963 from *R. sinicus* bats (Table 2). A Betacoronavirus isolate designated BM48 - 31/BGR/GU190215 was detected in *R. blasii* bats from Bulgaria. These bats are normally found in Africa, southern Europe, and western Asia. This isolate was evolutionarily placed between isolates YRa11/CN/KF569996 and Zhejiang2013/CN/KF636752 on the phylogenetic tree (Fig. 1). It shared 88.42% nucleotide sequence identity with the SARSr-CoV isolate Rs4231/CN/KY417146 from *R. sinicus* (Table 2). The BatCoV RaTG13/CN/MN996532 was found in *R. affinis* bats. It is evolutionarily closed to SARSr-CoVs (Fig. 1), and shared 97.83% with COVID-19 strain (SNU01/ROK/MT039890), 88.26 with human SARS-CoV strain (SZ16/HK/AY304488) (Table 2).

Isolates CMR66/CMR/MG693170, HKU9 - 1/CN/EF065513, and GCCDC1 - 356/CN/KU762338 were clustered together on the phylogenetic tree (Fig. 1). These isolates shared 78.03% - 96.24% nucleotide sequence identity with each other, and their hosts were all bats from the *Pteropodidae* family (flying fox).

In Alphacoronavirus, isolates 1B/CN/EU420137, AH2011/CN/KJ473795, 1A/CN/EU420138, HKU7 - 1/CN/DQ249226, and HKU8/CN/EU420139 were clustered together on the phylogenetic tree (Fig. 1) and shared 78.38% - 98.24% nucleotide sequence identity with each other. Their hosts were

all *Miniopterus* bats. Isolates AT1A – F1/GHA/KT25327 and KW2E – F151/GHA/KT253269 shared 95.76% nucleotide sequence identity. Their hosts were *Hipposideros* bats from Africa (Fig. 1).

**Bat Paramyxovirus.** Results of ParaFit and PACo analyses of nucleotide sequences of bat *cyt b* gene and paramyxovirus RNA polymerase large (*L*) gene indicated that paramyxoviruses and their bat hosts had a significant coevolutionary relationship (ParaFitGlobal = 874.11,  $P = 0.049$ ;  $m^2$  global value = 15.49537,  $P = 0.015$ ). Seven of the 36 individual host-parasite links were significant with a ParaFit1 or Parafit2 value of  $P \leq 0.05$ .

Paramyxoviruses were divided into two major branches on the phylogenetic tree. As most of them were unclassified (Fig. 2), they were divided into 4 groups (PG1-PG4) according to certain characteristics of their hosts. Isolates GB59 – 59/GHA/HQ660162, GB09670/GAB/HQ660156, GB59 – 30/GHA/HQ660161, GH19 – 140/GHA/HQ660153, GD2012/CN/KJ64165, and GB09682/GAB/HQ660157 (paramyxovirus group 1, PG1) were detected in *Hipposideros* bats (family: *Hipposideridae*) that are mainly distributed in Africa and Asia (Fig. 2). Isolates RCA – P18/RCA/HQ660152, CD273/DRC/HQ660122, GB1386/GAB/HQ660137, GB1237/GAB/HQ660140, and GH6/GHA/FJ971938 (paramyxovirus group 2, PG2) were detected in bats of the *Pteropodidae* family from Africa; these bats were not phylogenetically clustered together with the bats in this family from Asia, Oceania, and Australia (Fig. 2). Isolates KCR245H/CRC/JF828297, BR21/BRA/HQ660187, BR310/BRA/HQ660194, BR310/BRA/HQ660194, and BR190/BRA/HQ660190 (paramyxovirus group 3, PG3) were closely related. Their hosts are distributed in South and North America. The host of KCR245H/CRC/JF828297 was *Pteronotus parnellii* (family: *Mormoopidae*), and the hosts of the other four isolates were bats of the *Pteropodidae* family (Fig. 2). Seven closely related isolates GH36/GHA/FJ609192, 3 – 320/BGR/HQ660163, N78 – 14/GER/HQ660166, 6 – 43/BGR/HQ660164, NMS09 – 48/GER/HQ660165, Md – LN2012/CN/KJ641656, and NM98 – 46/GER/HQ660170 (paramyxovirus group 4, PG4) from Europe and Asia (Fig. 2) shared 70.13% – 97.32% nucleotide sequence identity with each other. Among them, isolate 6 – 43/BGR/HQ660164 was detected in *Myotis capaccinii* bats (family: *Vespertilionidae*) from Bulgaria, and isolate NMS09 – 48/GER/HQ660165 was found in *M. daubentoni* bats (family: *Vespertilionidae*) from Germany. These two isolates shared the highest nucleotide sequence identity.

Teviot virus (TeV), Tioman virus (TiV), and Menangle virus (MENV) were phylogenetically clustered in the same clade. These viruses are members of the genus *Pararubulavirus*, and their bat hosts were members of the *Pteropodidae* family. These bats are distributed in regions ranging from southeast Asia to northwest Oceania (Fig. 2). HeV, NiV, and several unclassified paramyxoviruses were clustered in one clade (Fig. 2). These results indicated that closely related bats carried closely related paramyxoviruses. Some bat hosts of HeV and NiV were found to be also infected by TiV and MENV (Fig. 2).

## Discussion

We tried to ensure that the sequences' length was long enough to elucidate the evolutionary relationship, while picking up diverse strains detected from different countries and species, and the sequences we choose in this study can represent the reported coronavirus and paramyxovirus sequences. The co-phylogenetic analysis results confirmed our hypothesis that coronavirus and paramyxovirus co-evolve with their host.

## Coronavirus

MERSr-CoVs are mostly found in *Pipistrellus*, *Tylonycteris*, *Hypsugo*, *Vespertilio*, and *Neoromicia* bats of the *Vespertilionidae* family. Bat MERSr-CoV isolates PDF – 2180/UG/KX574227 (bat host: *Pipistrellus hesperidus*), 5038/RSA/MF593268 (bat host: *Neoromicia capensis*), and PML – PHE1/RSA/KC869678 (bat host: *Neoromicia capensis*) were most closely related to human and camel MERS-CoVs (Fig. 1 and Table 1). Their pairwise nucleotide sequence similarity was > 91%, and all of them were detected in *Vespertilionidae* bats (*P. hesperidus* or *N. capensis*) from Africa. This observation suggests that these viruses might have been evolved from a common ancestor that infects different bat species in Africa. Isolates HKU5 – 1/CN/EF065509, HKU4 – 1/CN/EF065505, GX2012/CN/KJ473822, JPDB144/CN/KU182965, and GD2013/CN/KJ473820 share < 83% nucleotide identity with camel and human MERS-CoVs, and their hosts are distantly related to *N. capensis* bats. Isolates PML – PHE1/wRSA/KC869678, 5038/RSA/MF593268, NL13845/CN/MG021451, HKU25/CN/KX442565, SC2013/CN/KJ473821, 206645 – 40/IT/MG596802, and 206645 – 63/IT/MG596803 share > 85% nucleotide identity with camel and human MERS-CoV. Among them, the hosts of isolates SC2013/CN/KJ473821, HKU25/CN/KX442565, 206645 – 40/IT/MG596802 are close related to *N. capensis* bats, and the host of isolate 206645 – 63/IT/MG596803 is closely related to *P. hesperidus* bats. The high similarity (99.46%) in nucleotide sequences and host distribution areas (Italy) between isolates 206645 – 40/IT/MG596802 (bat host: *Hypsugo savii*) and 206645 – 63/IT/MG596803 (bat host: *Pipistrellus kuhlii*) suggests that they may be derived from inter-species transmission of the same strain of coronavirus in bats. We speculate that the ancestors of the hosts of bat MERSr-CoVs may be *Vespertilionidae* bats that are evolutionarily close to *N. capensis* and *P. hesperidus* bats distributed between West Asia and East Africa, and more attention should be pay on *N. capensis*.

SARSr-CoVs have been found in various *Rhinolophus* bats (Hu et al. 2017). Previous studies showed that *R. sinicus* bats are the natural hosts of SARS-CoVs (Lau et al. 2005, Ge et al. 2013, Yang et al. 2015). Although many SARSr-CoV were detected from *R. sinicus* and *R. ferrumequinum*, SARSr-CoVs from *R. ferrumequinum* shared lower nucleotide sequence identity with human SARS-CoVs than SARSr-CoVs from *R. sinicus*. *R. sinicus* bats are distributed in southern China, Nepal, northern India, and Vietnam, whereas *R. ferrumequinum* bats are present in northwest Africa and many European and Asian countries (Simmons 2005). The first outbreak of SARS occurred in Guangdong, China, where *R.*

*sinicus* but not *R. ferrumequinum* bats reside (Smith and Xie 2008). Isolate 16BO133/Korea/KY938558 shared 99.71% nucleotide sequence identity with isolate JL2012/KJ473811 and 99.68% with isolate JTMC15/KU182964. This high pairwise nucleotide sequence similarity among SARS-CoVs suggests that their hosts might be derived from the same ancestor. The SARSr-CoV isolate As6526/CN/KY417142 was detected in *Aselliscus stoliczkanus* bats of the Hipposiderinae family, which are phylogenetically related to *Rhinolophus* bats. Unexpectedly, *C. plicatus* bats (*Molossidae* family), which are distantly related to *Rhinolophus* bats, were found to carry the SARSr-CoV isolate Yunnan2011/CN/JX993988. Both isolates As6526/CN/KY417142 and Yunnan2011/CN/JX993988 were found in bats from Yunnan, China (Yang et al. 2013, Xu et al. 2016), they share 94.35% nucleotide identity, and their closest strains also found in Yunnan. These observations suggest that close evolutionary relationship and close roosting are required for inter-species transmission of viruses. The isolate BM48 – 31/BGR/GU190215 was detected in *R. blasii* bats from Bulgaria. It shared 88.42% nucleotide identity with the SARSr-CoV isolate Rs4231/CN/KY417146, which is evolutionarily placed between isolates Zhejiang2013/CN/KF636752 from *Hipposideros pratti* (Hipposiderinae family) and Rf1/CN/DQ412042 from *Rhinolophus ferrumequinum*. BM48 – 31/BGR/GU190215 is distantly related other SARSr-CoVs (Fig. 1), and showed lower nucleotide identity with human SARS-CoVs (Table 2). The observations suggest that the evolutionary relationship of viruses may be related to the geographical distribution distance and evolutionary distance of their hosts.

The BatCoV RaTG13/MN996532 is related to SARSr-CoV in the evolutionary tree (Fig. 1); they share 82% – 86% RdRp nucleotide identity. The COVID-19 shares 79.5% sequence identity with SARS-CoV and uses the same receptors, Angiotensin converting enzyme II (ACE2) receptors, as those for SARS-CoVs to enter human cells (Zhou and Yang 2020) BatCoV. RaTG13/CN/MN996532 shares 97.85% RdRp nucleotide identity with COVID-19 (SNU01/ROK/MT039890) and 96% at the whole genome level. There are more than 1100 bases that are different between BatCoV RaTG13/MN996532 and COVID-19 s, suggesting that BatCoV RaTG13/MN996532 may require one or more intermediate hosts to transmit to humans (Zhou and Yang 2020).

In this study, *Rhinolophus* bats were found to be infected mostly with SARSr-CoVs, while *Vespertilionidae* bats were mostly infected with MERSr-CoVs. These observations indicate host specificity of these coronaviruses. Most SARSr-CoVs were detected in bats from eastern Asia. It is unknown whether *Rhinolophus* bats in other regions of Asia or other continents carry coronaviruses that are related to SARS-CoVs. Whether climate and geography affect host specificity of viruses is also remain to be answered.

## Paramyxovirus

As described above, paramyxoviruses are divided into 4 groups, PG1 – PG4. The hosts of PG1 are mostly *Hipposideridae* bats from Africa and tropical Asia (Simmons 2005). The hosts of PG2 are mostly *Pteropodidae* bats in Africa, and those of PG3 are bat species closely related to members of *Phyllostomidae* and *Mormoopidae* families, which are distributed in Americas. The hosts of PG4 are *Vespertilionidae* bats distributed in Europe, Asia, and Africa (Fig. 2). These observations suggest that closely related viruses share similar host species and that these viruses were carried by an ancestor bat that migrated from one region to another and then diverged into different bat species.

NiV and HeV belong to the genus *Henipavirus* and cause highly fatal encephalitis in humans (Murray et al. 1995, Chua et al. 1999). *Pteropid* bats have been shown to be the natural reservoir of *Henipavirus* (Young et al. 1996, Halpin et al. 2000, Johara et al. 2001) and were speculated to be responsible for the outbreaks of *Henipavirus* in Malaysia, Australia, Singapore, Philippine, India and Bangladesh during the period of 1995–2015 (Murray et al. 1995, Chua et al. 1999, Chua et al. 2000, Hsu et al. 2004, Chadha et al. 2006, Arankalle et al. 2011, Ching et al. 2015). Isolates GB1237/GAB/HQ660140, GH6/GHA/FJ971938 and RCA – P10/RCA/HQ660149 CD356/DRC/HQ660126 were detected in *Eidolon helvum* and *Myonycteris torquata* bats that are evolutionarily close to *Henipavirus* (Fig. 2). Ghanaian bat virus (Ghana virus), which was recently identified as a *Henipavirus*, found in *E. helvum* bats in west Africa, might have arisen by zoonotic transmission (Drexler et al. 2009, Hayman et al. 2011). It has been shown that *P. giganteus* bats were the host of both NiV (a Henipavirus) and TiV (a Rubulavirus), and *P. alecto* bats were the host of both HeV (a Henipavirus) and MENV (a Rubulavirus). Whether an individual bat can harbor two or more different genus of paramyxovirus and whether there is genetic recombination between Henipavirus and Rubulavirus remain to be investigated.

Results of our study suggest that coronaviruses and paramyxoviruses have both host and geographical specificity. In general, different bat species reside in the same geographical areas were found to have close genetic relationships, and the viruses that they carried were also closely related. This may be the reason why hosts evolve as a consequence of environmental changes, and viruses evolve as their hosts are evolved. We also found evidence of distantly related bat species infected with closely related viruses (e.g., SARSr-CoVs As6526/CN/KY417142 and YN2018B/CN/MK211376; MERSr-CoVs 206645 – 40/IT/MG596802 and 206645 – 63/IT/MG596803) when they roosted close to each other. Furthermore, bat species in different families but close in evolution were found to be infected with related viruses. This observation suggests that inter-species or inter-family transmission may occur between genetically related or geographically close host species. Although endemic areas of both SARS and *Henipavirus* overlap highly with the distribution areas of their bat hosts, we have not found ancestors of MERS in the areas of MERS outbreaks. MERSr-CoVs that shared the highest nucleotide sequence identity with human and camel CoVs have been detected in *N. capensis* and *P. hesperidus* bats from Africa. However, most MERSr-CoVs carried by bats closely related to *N. capensis* shared higher nucleotide sequence identity with human and camel MERS-CoVs than those carried by bats closely related to *P. hesperidus*. We speculate that the ancestor bat host of MERS-CoVs was a member of *Vespertilionidae* related to *N. capensis* and distributed from east Africa to west Asia and Arabian

Peninsula. MERS-CoV infections were found to be ubiquitous in dromedaries in Africa and Arabian Peninsula, but zoonotic infections by MERS-CoVs were found to be confined in Arabian Peninsula (Chu et al. 2018). It is still possible to find sequence closer to COVID-19 from *R. affinis* related species.

In summary, results of this study showed that similar species of bats distribute in the same geographical areas, and that closely related bats harbor closely related viruses. Furthermore, our results imply that the overlap of home range is an important condition for virus inter-species transmission. Therefore, the similarity of bat viruses is related to both the evolution and geographical distribution of their hosts. In addition, the results suggest that the outbreak may be related to the distribution of species in the area. Continual surveillance of natural bat hosts of emerging viruses and endemic areas of viruses is warranted.

## Declarations

### Ethical approval statement

This study was conducted strictly in accordance with the Regulations for the Administration of Laboratory Animals (Decree No. 2, State Science and Technology Commission, People's Republic of China), and approved by the Guangdong Entomological Institute Animal Care Committee (No. GDEI-AE-2006001).

### Conflicts of interest

The authors declare no conflicts of interest.

### Author Contributions

LZ and JL conceived the ideas and designed the experiments. LZ did the morphological identification of species. JL performed the experiments and analyzed the data under the guidance of CZ. JL, CZ, and LZ wrote the paper.

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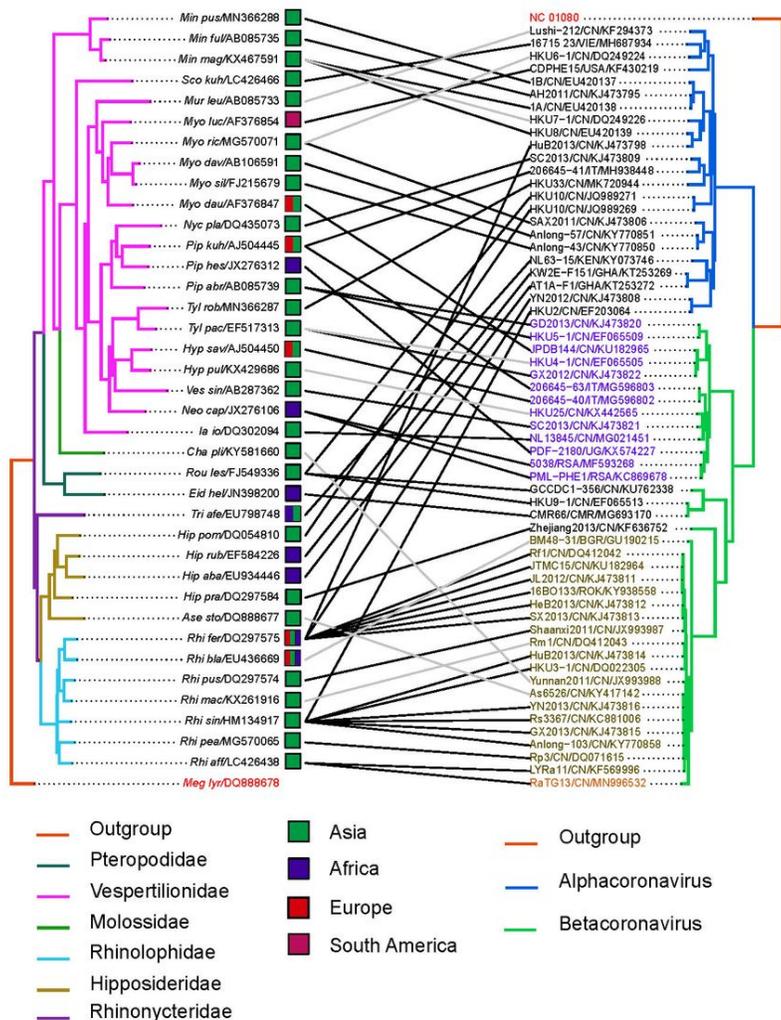
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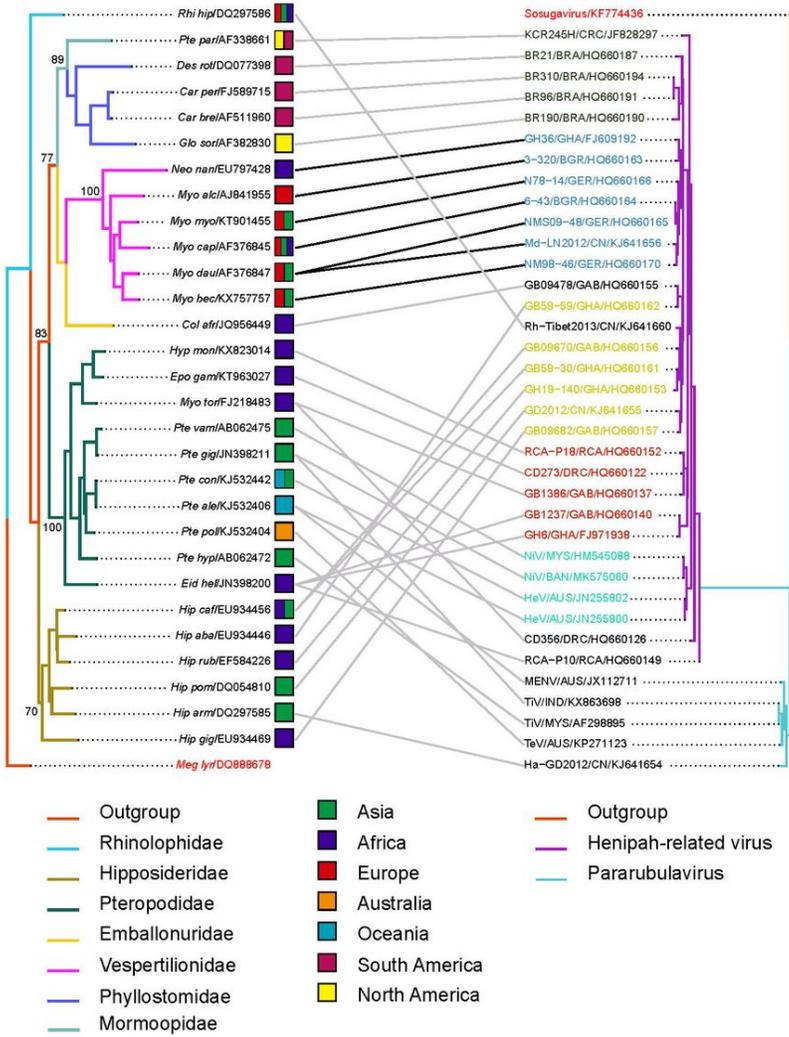
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## Figures



**Figure 1**

Tanglegram of cophylogenetic relationship between bat hosts and coronaviruses. Maximum-likelihood phylogenies for coronaviruses (right) and their bat hosts (left) are shown with bootstrap support values  $\geq 50$  labeled on main branches. Outgroups were used to root the phylogenetic tree of bat hosts and coronaviruses. Black lines denote significant cospeciation links between coronaviruses and their hosts (ParaFit tests  $P \leq 0.05$ ), and gray lines denote non-significant links. Information on host geographical distribution was derived from Simmons (2005).



**Figure 2**

Tanglegram of cophylogenetic relationships between bat hosts and paramyxoviruses. Maximum-likelihood phylogenies for bat hosts (left) and paramyxoviruses (right) are shown with bootstrap support values  $\geq 50$  labeled on main branches. Outgroups were used to root the phylogenetic tree of bat hosts and paramyxoviruses. Black lines denote significant cospeciation links between paramyxoviruses and their hosts (ParaFit tests  $P \leq 0.05$ ), and gray lines denote non-significant links. Information on host geographical distribution was derived from Simmons (2005).