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

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Research article

Keywords: coevolution, coronavirus, paramyxovirus, SARS, MERS, Hendra virus, Nipha virus, COVID-19

Posted Date: September 15th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-21963/v2>

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Version of Record: A version of this preprint was published at BMC Ecology and Evolution on July 29th, 2021. See the published version at <https://doi.org/10.1186/s12862-021-01878-7>.

Abstract

Background: Bat-borne viruses are relatively host specific. In this study, we investigated the coevolution of coronavirus and paramyxovirus with their bat hosts. Published nucleotide sequences of the RNA-dependent RNA polymerase (RdRp) gene of 60 coronaviruses isolated from 37 bat species, the RNA polymerase large (L) gene of 36 paramyxoviruses isolated from 29 bat species, and the cytochrome B (*cytB*) gene of 35 bat species were analyzed for coevolution signals. Each coevolution signal detected was tested and verified by the ParaFit and PACo functions in the R program.

Results: Significant coevolution signals were detected in coronaviruses and paramyxoviruses and their bat hosts, and closely related bat hosts were found to carry closely related viruses.

Conclusions: Our results suggest that similar geographical distribution and close phylogenetic relationship are requisites for inter-species transmission of viruses.

Background

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, Middle East respiratory syndrome coronavirus, severe acute respiratory syndrome coronavirus-2) (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).

Coronaviruses are taxonomically placed in the subfamily *Coronavirinae* under the family *Coronaviridae* (International Committee on Taxonomy of Viruses). Bats coronaviruses (BatCoVs) have been shown to be responsible for the 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 probably the current COVID-19 (Zhou and Yang 2020). They cause serious respiratory and intestinal symptoms with substantial mortality rates (Gralinski and Baric 2015).

Some paramyxoviruses such as Hendra virus (HeV) and Nipah virus (NiV) are highly pathogenic zoonoses. Both viruses belong to the genus *Henipavirus* of the family *Paramyxoviridae* (International Committee on Taxonomy of Viruses) and have been detected in flying-fox bats (*Pteropus spp.*) (Smith et al. 2011; Anderson et al. 2019). *Henipavirus* causes severe symptoms associated with high mortality rates in humans and livestock. HeV was first detected 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). NiV was first detected in Malaysia in 1999 during the outbreak of encephalitis and respiratory illness in pig farmers. In the past few years, sporadic outbreaks of HeV and NiV have occurred in Oceania and Southeast Asia (Harit et al. 2006; Mahalingam et al. 2012; Sharma et al. 2019).

Both coronaviruses and paramyxoviruses have a certain degree of host specificity. Host-parasite specificity has also been observed in malaria parasites (Ricklefs et al. 2004), bat flies (Nikon et al. 2011), and bacteria (Lei and Olival 2014). We hypothesized that the host specificity of coronaviruses and paramyxoviruses is a result of coevolution with their hosts, rendering them to efficiently proliferate in the hosts. To investigate the coevolution of coronavirus and paramyxovirus with their bat hosts, we compared the nucleotide sequences of the cytochrome B (*cytB*) gene of 61 bat species with those of the RNA dependent RNA polymerase (RdRp) gene of 60 coronaviruses and the RNA polymerase large (L) gene of 36 paramyxoviruses. Significant coevolution signals were detected in coronaviruses and paramyxoviruses and their bat hosts.

Methods

Phylogenetic Analysis

The database of Bat-associated Viruses (DBatVir, <http://www.mgc.ac.cn/DBatVir/>) (Chen et al. 2014) contains information on various bat-associated viruses, including genome size, lengths of identified genes, data and place (city and country) of isolation, names of the viruses, and GenBank accession numbers of nucleotide sequences of the entire genome or individual genes. With the “browse by virus” function, 60 coronavirus and 36 paramyxovirus isolates with all the aforementioned information available were found. As this database does not contain actual sequences, nucleotide sequences of the selected viral isolates were downloaded from the GenBank. These sequences included those of the RNA dependent RNA polymerase (RdRp) gene (2734 bp) of the 60 coronavirus isolates and the RNA polymerase large (L) gene (559 bp) of the 36 paramyxovirus isolates. The 60 coronavirus isolates were derived from 37 different bat species, and the 36 paramyxovirus isolates were derived from 29 different bat species. Among them, 5 bat species including *Myotis daubentonii*, *Eidolon helvum*, *Hipposideros abae*, *Hipposideros ruber*, and *Hipposideros pomona* were found to harbor both coronavirus and paramyxovirus. Altogether, 61 different species of bats were identified to be the host of the coronavirus and paramyxovirus isolates examined in this study. As the cytochrome B (*cytB*) gene is one of the most conserved gene in bats, its sequence was used for coevolution analyses. The *cytB* sequences of 59 of the 61 bat species were downloaded from the GenBank. Since the *cytB* gene sequences of *Miniopterus pusillus* and *Tylonycteris robustula*, that were the hosts of coronavirus isolates 1B/CHN/EU420137 and HKU33/CHN/MK720944, respectively, were not available, they were determined in this study. These two bat species were captured from Menghai, Yunnan and Kau O Bat Cave, Macau, respectively. Anal swabs of *Miniopterus pusillus* and a small portion of the patagiums of *Tylonycteris robustula* were obtained. The captured bats were released back to their roosts after sampling. DNA was isolated from these samples and used as the template for amplification by polymerase chain reaction (PCR) of a portion (1140 bp) of the *cytB* gene with primers L14727ag (5'- ATGATATGAAAAACCATCGTTG) and H15915ag (5'- TTTCCNTTTCTGGTTTACAAGAC) (Guillén-Servent and Francis 2006). PCR conditions were as follows: 94°C for 3 min, followed by 20 cycles of 94°C for 20s, 46°C to 52°C (+0.3°C/cycle) for 30s, and 72°C for 90s and 30 cycles of 94°C for 20s, 60°C for 30s, 72°C for 90s and then maintained at 72°C for 10 min.

The PCR products were sequenced, and the sequences thus obtained have been deposited in the Genbank with accessing numbers MN366287 and MN366288.

To analyze the nucleotide sequences, they were aligned with Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo>) (Sievers and Higgins 2014). The outgroup sequences included in the sequence alignments were those of Turkey COV/NC_01080 coronavirus, Sosugavirus/KF774436 paramyxovirus, and *Megaderma lyra*/DQ888678 bat. Maximum-likelihood phylogenetic trees were constructed using 1000 bootstraps with the raxmlGUI program (Silvestro and Michalak 2012). The GTR+I+G model for analysis of nucleotide substitutions was established 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 was determined using the global-fit function of the software package ParaFit, which tests the hypothesis of coevolution between hosts and their parasites by a global test of coevolution and a test on each host-parasite (H-P) link (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 software package ape (Paradis et al. 2004). ParaFit analyses were performed with 999 permutations for both Global and Individual H-P link tests. Each individual host-virus link was considered as significant when its ParaFit 1 or Parafit 2 P-value was ≤ 0.05 (Lei and Olival 2014). To verify the results, each phylogenetic signal was tested using the software package Procrustean Approach to Cophylogeny (PACo) (Balbuena et al. 2013), which differs from ParaFit in that the virus matrix was 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 significance of coevolution of each host–virus link (Singh et al. 2017). Cophylogenetic trees were generated using the “cophylo” function of the R package phytools (Revell 2011).

Results

Phylogenetic relationship of bat coronavirus isolates with their bat hosts. Results showed that the bat coronavirus (BatCoV) isolate RaTG13/CN/MN996532 from *Rhinolophus affinis* found in Yunnan Province, China is on the same branch of the phylogenetic tree as the 18 human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolates examined, suggesting that it is a relative of SARS-CoV-2 (Figure 1). The following 18 BatCoV isolates are clustered on the same branch of the phylogenetic tree as the six human severe acute respiratory syndrome coronavirus (SARS-CoV) isolates analyzed, suggesting that they are phylogenetic relatives: Rf1/CHN/DQ412042, JTMC15/CHN/KU182964, JL2012/CHN/KJ473811, 16BO133/ROK/KY938558, HeB2013/CHN/KJ473812, SX2013/CHN/KJ473813, Shaanxi2011/CHN/JX993987, Rm1/CHN/DQ412043, HuB2013/CHN/KJ473814, HKU3–1/CHN/DQ022305, Yunnan2011/CHN/JX993988, As6526/CHN/KY417142, YN2013/CHN/KJ473816, Rs3367/CHN/KC881006, GX2013/CHN/KJ473815, Anlong–103/CHN/KY770858, Rp3/CHN/DQ071615, and LYRa11/CHN/KF569996. The BatCoV isolate

BM48-31/BGR/GU190215 from *Rhinolophus blasii* living in Bulgaria is found to be distantly related to the six human SARS-CoV isolates as it is located alone on a branch of the phylogenetic tree (Figure 1). The following 13 BatCoV isolates were clustered on the same branch of phylogenetic tree as the six human Middle East respiratory syndrome coronavirus (MERS-CoV) isolates examined, suggesting that they are evolutionarily close to each other: JPDB144/CHN/KU182965, HKU4-1/CHN/EF065505, GX2012/CHN/KJ473822, HKU5-1/CHN/EF065509, GD2013/CHN/KJ473820, PDF-2180/UGA/KX574227, 5038/RSA/MF593268, PML-PHE1/RSA/KC869678, SC2013/CHN/KJ473821, HKU25/CHN/KX442565, NL13845/CHN/MG021451, 206645-40/ITA/MG596802, and 206645-63/ITA/MG596803 (Figure 1).

Coevolution of bat coronaviruses with their bat hosts. Analyses of all bat *cytB* gene sequences and all BatCoV RdRp gene sequences as a whole by the Global test function of ParaFit and PACo showed evidence of coevolution between BatCoV isolates and their bat hosts (ParaFitGlobal = 390.8896, $P = 0.001$; m^2 global value = 57.136, $P \leq 0.001$). When each individual sequence was analyzed by the Individual H-P link test function of the ParaFit software package, 51 of the 60 BatCoV isolates were found to have a significant coevolution relationship (link) with their bat hosts with a ParaFit1 or ParaFit2 P value ≤ 0.05 .

BatCoV isolates examined in this study are divided into alpha and beta groups (Figure 1). In the alphacoronavirus group, BatCoV isolates 1B/CHN/EU420137, AH2011/CHN/KJ473795, 1A/CHN/EU420138, HKU7-1/CHN/DQ249226 and HKU8/CHN/EU420139 are related and are all derived from *Miniopterus* bats. Isolates SAX2011/CHN/KJ473806, Anlong-57/CHN/KY770851, and Anlong-43/CHN/KY770850 are also related and are all from *Myotis* bats. Similarly, isolates KW2E-F151/GHA/KT253269 and AT1A-F1/GHA/KT253272 are related and are all from *Hipposideros* bats. These observations suggest host specificity of these BatCoV isolates. The following BatCoV isolates are closely related but are from different species of bats of the same family (*Vespertilionidae*): Lushi-212/CHN/KF294373 (from *Murina leucogaster*), 16715 23/VIE/MH687934 (from *Scotophilus kuhlii*), HKU6-1/CHN/DQ249224 (from *Myotis ricketti*), and CDPHE15/USA/KF430219 (from *Myotis lucifugus*) (Figure 2). This result suggests that some BatCoV isolates have a less stringent host specificity than others.

In this study, BatCoV isolates that are related to human SARS-CoVs are referred to as SARSr-CoVs, and those that are related to human MERS-CoVs are referred to as MERSr-CoVs. These viruses are clustered on the betacoronavirus branch of the phylogenetic tree (Figure 1). Most bat SARSr-CoV isolates are found in members of the *Rhinolophidae* bat family, and most bat MERSr-CoV isolates are derived from members of the *Vespertilionidae* bat family. BatCoV isolates CMR66/CMR/MG693170, HKU9-1/CHN/EF065513, and GCCDC1-356/CHN/KU762338 with 78.03 - 96.24% RdRp gene sequence identity are clustered together on the same branch of the phylogenetic tree and are all from members of the *Pteropodidae* bat family (Figure 2).

BatCoV isolates 206645-40/IT/MG596802 and 206645-63/IT/MG596803 are related with 99.46% RdRp gene sequence and are found in *Hypsugo savii* and *Pipistrelle kuhlii*, respectively, in Italy (Figure 2). The

bat SARSr-CoV isolate 16B0133/ROK/KY938558 found in *Rhinolophus ferrumequinum* in South Korea is evolutionarily close to BatCoV isolates JL2012/KJ473811 (99.71% RdRp sequence identity) and JTM15/KU182964 (99.68% RdRp sequence identity) found in the same species (*Rhinolophus ferrumequinum*) of bats in Jilin Province, China (Xu et al 2016) (Figure 2). The BatCoV isolate As6526/CHN/KY417142 is found in *Aselliscus stoliczkanus* (family *Hipposideridae*) that are evolutionarily close to *Rhinolophidae* bats (Figure 2). BatCoV isolate Yunnan2011/CHN/JX993988 found in Yunnan Province, China is from *Chaerephon plicatus* (family *Molossidae*) that are distantly related to *Rhinolophidae* bats (Figure 2).

Coevolution of bat paramyxoviruses with their bat hosts. Analyses of all bat *cytB* gene sequences and all paramyxovirus RNA polymerase large (L) gene sequences as a whole by the Global test function of ParaFit and PACo showed evidence of coevolution between bat paramyxovirus isolates and their bat hosts (ParaFitGlobal = 874.11, $P = 0.049$; m^2 global value = 15.49537, $P = 0.015$). When each individual sequence was analyzed by the Individual H-P link test function of ParaFit, 7 of the 36 bat paramyxovirus isolates were found to have a significant host-parasite coevolution relationship (link) with a ParaFit1 or Parafit2 p value ≤ 0.05 .

In this study, we classified unidentified bat paramyxovirus isolates into four groups PG1- PG4 according to their host specificity. PG1 paramyxovirus isolates GB59-59/GHA/HQ660162, GB09670/GAB/HQ660156, GB59-30/GHA/HQ660161, GH19-140/GHA/HQ660153, GD2012/CHN/KJ64165, and GB09682/GAB/HQ660157 are closely related and are all derived from *Hipposideros* bats (family: *Hipposideridae*) (Figure 3). The following PG2 paramyxovirus isolates are from *Pteropodidae* bats and are closely related: RCA-P18/RCA/HQ660152, CD273/DRC/HQ660122, GB1386/GAB/HQ660137, GB1237/GAB/HQ660140, and GH6/GHA/FJ971938 (Figure 3). Isolates KCR245H/CRC/JF828297, BR21/BRA/HQ660187, BR310/BRA/HQ660194, BR310/BRA/HQ660194, and BR190/BRA/HQ660190 that belong to paramyxovirus group 3 (PG3) are closely related (Figure 3). The host of isolate KCR245H/CRC/JF828297 is *Pteronotus parnellii* bat (family: *Mormoopidae*), and the hosts of the other four isolates are bats of the *Pteropodidae* family, including *Desmodus rotundus*, *Carollia perspicillata*, *Carollia brevicauda*, *Glossophaga soricina* (Figure 3). Seven closely related isolates, including GH36/GHA/FJ609192, 3-320/BGR/HQ660163, N78-14/GER/HQ660166, 6-43/BGR/HQ660164, NMS09-48/GER/HQ660165, Md-LN2012/CHN/KJ641656, and NM98-46/GER/HQ660170 (paramyxovirus group 4, PG4), are found in members of the *Vespertilionidae* bat family (Figure 3). Identified bat paramyxoviruses including Teviot virus (TeV), Tioman virus (TiV), and Menangle virus (MENV) are members of the genus *Pararubulavirus*, their bat hosts are members of the *Pteropodidae* family. The hosts of *Henipavirus*, NiV and HeV are from *Pteropodidae* family as well (Figure 3).

The bat hosts of PG1 paramyxoviruses are distributed mainly in Africa and Asia, and those of PG2 paramyxoviruses are mostly living in Africa. PG3 paramyxoviruses are mostly found in bats in south and north America. The bat hosts of PG4 paramyxoviruses are distributed in Asia, Africa, and Europe. The bat hosts of *Pararubulavirus* and *Hennipahvirus* are found in areas from Asia to Oceania (Figure 3).

Discussion

In this study, we investigated the coevolution relationship between bats and their viral parasites: coronaviruses and paramyxoviruses. These two groups of viruses were chosen because they have been shown to be zoonotic (Rizzo et al 2017). The sequences of the RNA-dependent RNA polymerase (RdRP) gene of 60 bat coronavirus (BatCoV) isolates, the RNA polymerase large (L) gene of 36 paramyxovirus isolates, and the cytochrome B (cytB) gene of 61 bat species were used to build phylogenetic trees. ParaFit analyses were then performed to determine the relationship between coronavirus and bat genetic trees and between paramyxovirus and bat genetic trees. Both ParaFit Global and ParaFit Individual tests were performed. In the Global test, both groups of the viruses were found to have a significant coevolution relationship with their bat hosts. In the Individual test, 51 (85%) of the 60 BatCoV isolates and 7 (19%) of the 36 paramyxovirus isolates had a significant coevolution relationship with their bat hosts.

ParaFit analyses also revealed that closely related BatCoV isolates are found in closely related bat species (Figure 2). One example of such observation is that closely related BatCoV isolates 1B/CHN/EU420137, AH2011/CHN/KJ473795, 1A/CHN/EU420138, and HKU8/CHN/EU420139 are found in *Miniopterus pusillus*, *Miniopterus fuliginosus*, and *Miniopterus magnater* that are very close to each other. Another example is the coevolution relationship between closely related bats including *Tylonycteris pachypus*, *Hypsugo savii*, *Vespertilio sinensis*, *Neoromicia capensis*, and *Laio* and the following BatCoV isolates: GX2012/CHN/KJ473822, 206645-40/ITA/MG596802, SC2013/CHN/KJ473821, NL13845/CHN/MG021451, 5038/RSA/MF593268, and PML-PHE1/RSA/KC869678. Similar coevolution relationships are found in closely related bat species *Rhinolophus ferrumequinum*, *Rhinolophus blasii*, *Rhinolophus pusillus*, *Rhinolophus macrotis*, *Rhinolophus sinicus*, *Rhinolophus pearsonii*, and *Rhinolophus affinis* and the following BatCoV isolates: RF1/CHN/DQ412042, JTM15/CHN/KU182964, JL2012/CHN/KJ473811, 16B0133/ROK/KY938558, HeB2013/CHN/KJ473812, SX2013/CHN/KJ473813, Shaanxi2011/CHN/JX993987, HuB2013/CHK/KJ473814, HKU3-1/CHN/DQ022305, YN2013/CHK/KJ473816, Rs3367/CHN/KC881006, GX2013/CHN/KJ473815, Anlong-103/CHN/KY770858, Rp3/CHN/DQ071615, LYRa11/CHN/KF569996, and RaTG13/CHN/MN996532 (Figure 2). For paramyxoviruses, closely related bat species *Neoromicia nanus*, *Myotis alcathoe*, *Myotis myotis*, *Myotis capaccinii*, *Myotis daubentonii*, and *Myotis bechsteinii* are found to carry the following closely related isolates: GH36/GHA/FJ609192, 3-320/BGR/HQ660163, N78-14/GER/HQ660166, 6-43/BGR/HQ660164, NMS09-48/GER/HQ660165, LN2012/CHN/KJ641656, and NM98-46/GER/HQ660170 (Figure 3).

As mentioned above, 85% (51/60) of BatCoV isolates but only 19% (7/36) of paramyxovirus isolates were found to have a significant coevolution relationship with their bat hosts by the ParaFit Individual test. Since significant coevolution was found in both groups of the viruses by the ParaFit Global test, this low positive individual link rate in paramyxoviruses may be due to the small sample size. However, this observation may suggest that BatCoVs adapt to their bat hosts more readily than paramyxoviruses. This possibility is supported the fact that coronaviruses are more commonly found in bats than paramyxoviruses (Liang et al. 2017). Further studies are warranted to test this hypothesis.

We postulate that that evolutionary relationship and close habitat of bat species contribute to inter-species transmission of viruses. One observation supporting this hypothesis is that bat coronavirus isolates 206645–40/IT/MG596802 (bat host: *Hypsugo savii*) and 206645–63/IT/MG596803 (bat host: *Pipistrellus kuhlii*) share 99.46% nucleotide identity in their RdRp gene and are found in bats living in the same geographical area, Italy. In addition, bat coronavirus isolates 16B0133/ROK/KY938558, JL2012/KJ473811, and JTMC15/KU182964 are highly related with >99.6% nucleotide identity in the RdRp gene and are found in bats distributing in areas near each other, including Jilin Province, China (for isolates JL2012/KJ473811 and JTMC15/KU182964) and South Korea (for isolate 16B0133/ROK/KY938558) that is close to north China.

Most bat SARSr-CoV isolates are from *Rhinolophus* bats, but isolates As6526/CHN/KY417142 and Yunnan2011/CHN/JX993988 are found in *Aselliscus stoliczkanu* and *Chaerephon plicatus*, respectively. Although these two bat species are phylogenetically far apart, they live in the same geographical area, Yunnan Province, China. This observation suggests that distantly related bats in the same geographical location may carry closely related BatCoVs, leading to inter-species transmission of the viruses.

We also hypothesized that coevolution of distantly related viruses with hosts living in different geographical areas is unlikely to occur. This hypothesis is supported by the observation that the BatCoV isolate BM48–31/BGR/GU190215 is distantly related to other bat SARSr-CoV isolates (Figure 2). Its host, *Rhinolophus blasii*, lives in west Asia, north Africa, and south Europe (Simmons 2005) that are geographically distant from Yunnan Province, China, where many of the other BatCoV isolates examined in this study originated (Hu et al. 2017). Results of previous studies suggest that *Rhinolophus sinicus* is the natural hosts of human SARS-CoVs (Lau et al. 2005, Ge et al. 2013, Yang et al. 2015). Many bat SARSr-CoVs are detected in *Rhinolophus sinicus* and *Rhinolophus ferrumequinum* that distributed in Asia, Africa, and Europe. However, SARSr-CoVs that are highly related to human SARS-CoVs have not been found in *Rhinolophus ferrumequinum* that live in Africa and Europe. The BatCoV isolate LUX16_A_24/LUX/KY502395 found in *Rhinolophus ferrumequinum* in Luxembourg, Europe is distantly related to the SARS-CoV isolates found in humans and civets (Pauly et al. 2017).

For paramyxoviruses, *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 are speculated to be responsible for its outbreak in Malaysia, Australia, Singapore, Philippines, 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). Several species of *Pteropid* bats, including *Pteropus alecto*, *Pteropus conspicillatus*, *Pteropus giganteus*, and *Pteropus vampyrus*, have been found to carry *Henipaviruses*. These bats live in southeast Asia and Oceania (Simmons 2005), where *Henipavirus* pandemic occurred.

Most human SARS-CoV isolates examined in this study are derived from *Rhinolophus sinicus* in Yunnan Province, China. As *Rhinolophus sinicus* is found only in China, Nepal, Vietnam, and north India (Simmons 2005), SARS-CoV outbreak has not occurred in other places such as Europe, Africa, Oceania, or America. SARS-CoV-2 is responsible for the COVID-19 pandemic (International Committee on Taxonomy

of Viruses). In this study, the BatCoV isolate RaTG13/CHN/MN996532 is found to be most close to SARS-CoV-2 with >97% nucleotide identity in the RdRp gene and 96% identity at the whole genome level. There are approximately 1100 bases that are different between the genomes of BatCoV RaTG13/MN996532 and SARS-CoV-2s, suggesting that BatCoV RaTG13/MN996532 requires at least one intermediate host to transmit to humans (Zhou and Yang 2020). As the host of isolate RaTG13/MN996532 is *Rhinolophus affinis* residing in Yunnan Province, China, it has been speculated that SARS-CoV-2 is derived from *Rhinolophus* bats roosting in areas near Yunnan Province, China, such as southwest China, Myanmar, Laos, Vietnam, or other southeast Asian countries (Latinne et al 2020).

Divergence of bats can be traced back to tens of million years ago (Teeling et al. 2005, Agnarsson et al. 2011). It has been estimated that coronaviruses diverged tens of thousand years ago (Woo et al. 2012). This difference may be due to the fact that the genome of coronaviruses is RNA that is more prone to mutations than DNA. It has also been estimated that coronaviruses have been infecting birds or bats for tens of million years; this would confer the opportunity for coevolution of coronaviruses with their hosts (Wertheim et al. 2013).

Conclusion

We found evidence suggesting that both coronavirus and paramyxovirus coevolve with their bat hosts. This coevolution is more likely to occur between these viruses and their bat hosts that reside in the same or neighboring geographical areas.

Abbreviations

RdRp: RNA-dependent RNA polymerase; L: RNA polymerase large gene; *cytB*: cytochrome B; BatCoV: bat coronavirus; HeV: Hendra virus; NiV: Nipah virus; TeV: Teviot virus; TiV: Tioman virus; MENV: Menangle virus; H-P: host-parasite; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SARS-CoV: severe acute respiratory syndrome coronavirus; MERS-CoV: Middle East respiratory syndrome coronavirus; SARSr-CoV: BatCoV related to human SARS-CoV; MERSr-CoV: BatCoV related to human MERS-CoV.

Declarations

Ethics approval and consent to participate

This study was conducted 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).

Consent for publication

Not applicable

Availability of data and materials

The nucleotide sequences of the *cytB* gene of *Miniopterus pusillus* and *Tylonycteris robustula* bats are available at GenBank with accession numbers MN366287 and MN366288

Competing interests

The authors declare no conflicts of interest.

Funding

This work was supported by grants from the GDAS Special Project of Science and Technology Development (2018GDASCX-0107) and the Guangdong Provincial Science and Technology Program (2018B030324001).

Authors' Contributions

LZ and JL conceived the ideas and designed the experiments. LZ performed morphological identification of bats. JL performed experiments and analyzed data under the guidance of CZ. JL and LZ wrote the paper. All authors have read and approved the final manuscript.

Acknowledgements

We thank Professors Xinglou Yang, Yi-Hsuan Pan, and Chao-Hung Lee for valuable advices in this study.

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Figures

Figure 1

Phylogenetic analysis of the 2734-bp RNA-dependent RNA polymerase (RdRp) gene of coronaviruses isolated from humans and various species of bats.

Figure 2

Tanglegram of cophylogenetic relationship between bat hosts and coronaviruses. Black lines denote significant coevolution links between coronaviruses and their hosts (ParaFit tests $P \leq 0.05$), and gray lines denote non-significant links. Different groups of coronaviruses and bat species with significant coevolution links are marked with boxes in different colors. Information on host geographical distribution was derived from Simmons (2005).

Figure 3

Tanglegram of cophylogenetic relationships between bat hosts and paramyxoviruses. Black lines denote significant coevolution links between paramyxoviruses and their hosts (ParaFit tests $P \leq 0.05$), and gray lines denote non-significant links. Different groups of paramyxoviruses and bat species with significant coevolution links are marked with boxes in different colors. Information on host geographical distribution was derived from Simmons (2005).