



## Extensive diversity of coronaviruses in bats from China

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### ABSTRACT

To help reveal the diversity and evolution of bat coronaviruses we collected 1067 bats from 21 species in China. A total of 73 coronaviruses (32 alphacoronaviruses and 41 betacoronaviruses) were identified in these bats, with an overall prevalence of 6.84%. All newly-identified betacoronaviruses were SARS-related Rhinolophus bat coronaviruses (SARSr-Rh-BatCoV). Importantly, with the exception of the S gene, the genome sequences of the SARSr-Rh-BatCoVs sampled in Guizhou province were closely related to SARS-related human coronavirus. Additionally, the newly-identified alphacoronaviruses exhibited high genetic diversity and some may represent novel species. Our phylogenetic analyses also provided insights into the transmission of these viruses among bat species, revealing a general clustering by geographic location rather than by bat species. Inter-species transmission among bats from the same genus was also commonplace in both the alphacoronaviruses and betacoronaviruses. Overall, these data suggest that high contact rates among specific bat species enable the acquisition and spread of coronaviruses.

### 1. Introduction

Coronaviruses (CoVs; family *Coronaviridae*) are enveloped positive-sense, single-stranded RNA viruses with the largest genomes (25–31 kb) among known RNA viruses (de Groot et al., 2011). Based on genome-scale phylogenies the known CoVs are classified into 30 species within four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* (ICTV, 2017). Coronaviruses can infect humans, other mammals, and birds, causing respiratory, enteric, hepatic, and neurological diseases of varying severity (Masters and Perlman, 2013). Coronaviruses are well known globally due to the emergence of severe acute respiratory syndrome (SARS) during 2002–2003 caused by a previously unknown CoV

(Ksiazek et al., 2003; Peiris et al., 2003). Subsequently, other two human CoVs (NL63 and HKU1) causing respiratory disease were identified (van der Hoek et al., 2004; Woo et al., 2005). Strikingly, the Middle East respiratory syndrome (MERS) that emerged in 2012 and characterized by a higher mortality than SARS was also caused by a previously unknown CoV (Bermingham et al., 2012; Zaki et al., 2012). The ongoing emergence of these CoVs in humans means that CoVs will likely remain a key public health threat for the foreseeable future.

Since the discovery of SARS-CoV in Himalayan palm civets (Guan et al., 2003), intense effort has been directed toward identifying and characterizing coronaviruses from animals globally. Consequently, a number of CoVs have been identified in a diverse range of vertebrates including domestic and wild mammals, and birds (Poon et al., 2005;

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Wang et al., 2015; Woo et al., 2012). Bats (order Chiroptera), with more than 1240 species, have remarkable species diversity, and comprise more than 20% of living mammalian species (Nowak, 1994). The discovery of SARS-related CoV in *Rhinolophus* horseshoe bats in China in 2005 (Lau et al., 2005; Li et al., 2005) attracted the global attention to these mammals, such that diverse Alpha- and Beta-CoVs have now been identified in a variety of bats globally over the past decade (Corman et al., 2013, 2014, 2015; Drexler et al., 2014; He et al., 2014; Huang et al., 2016; Smith et al., 2016; Woo et al., 2012). More importantly, due to the close relationship between CoVs in bats and those causing human infections, it is believed that bats are the original source of human CoVs including SARS-CoV and MERS-CoV (Corman et al., 2015; Ge et al., 2013; Huynh et al., 2012; Ithete et al., 2013; Tao et al., 2017). Due to their high diversity and biological and ecological characteristics that potentially facilitate virus maintenance and transmission, bats likely harbor a large number of viruses, some of which may then jump to other species (Balboni et al., 2012). Hence, more effort is needed to identify and characterize the currently unrecognized CoVs that circulate in bats.

At least 120 bat species are found in China, mainly distributed in the eastern, central, and southern regions of that country (Zhang et al., 1997). Herein we report novel and diverse CoVs and SARS-related CoVs identified in *Rhinolophus*, *Miniopterus*, *Murina* and *Myotis* spp. bats sampled from several geographic regions of China. In addition, we inferred their genomic characteristics and evolutionary relationships with known viruses and their hosts.

## 2. Results

### 2.1. Bats collected and prevalence of CoVs

During 2012–2015 a total of 1067 bats were collected from five caves in five counties from Guizhou, Henan, and Zhejiang provinces (Fig. 1, Table 1 and S1). After morphological examination and sequence analysis of the mitochondrial cytochrome *b* (*mt-cyt b*) gene,

these bats were assigned to 21 species. The species and their abundance varied among regions, with *Miniopterus schreibersii* (47%) in Guizhou, *Rhinolophus ferrumequinum* (45%) and *R. pusillus* (32%) in Henan, and *R. monoceros* (41%) and *R. sinicus* (50%) in Zhejiang as the predominant species. Notably, only *R. pusillus* bats were found in all five regions.

Using RT-PCR targeting a conserved fragment of the RdRp (RNA-dependent RNA polymerase) gene of CoV as described previously (Lau et al., 2005; Wang et al., 2015), viral RNA was identified in a total of 73 bat fecal samples, with an overall detection rate of 6.84% (Table 1). Phylogenetic analysis revealed that all these viral sequences were clearly closely related to coronaviruses. Among the predominant bat species, CoV prevalence was high in *R. monoceros* (28/119, 23.53%) and *M. schreibersii* (16/198, 8.08%), but was lower in *R. sinicus* (8/219, 3.65%), *R. ferrumequinum* (2/183, 1.09%) and *R. pusillus* (1/154, 0.64%).

Of the 73 newly-identified CoVs, 32 belong to alphacoronaviruses and 41 to betacoronaviruses. To better characterize these newly-identified bat CoVs, the complete viral RdRp gene sequence was obtained from 65 (89%) of the viral RNA positive bat samples. In addition, 5 complete and 4 near-complete viral genome sequences were successfully obtained from CoV positive samples.

### 2.2. Newly-identified SARS-related *Rhinolophus* bat coronaviruses in bats

Genetic analysis of the conserved domains in the replicase poly-protein pp1ab – ADP-ribose 1-phosphatase (ADRP), nsp5 (3CLpro), nsp12 (RdRp), nsp13 (Hel), nsp14 (ExoN), nsp15 (NendoU) and nsp16 (O-MT) – revealed that newly-identified bat betacoronaviruses shared more than 90% amino acid sequence identity with *Severe acute respiratory syndrome-related coronavirus* (SARSr-CoV) (Table S2) and clustered together in a phylogenetic analysis (Fig. 2). Hence, these data suggest that these bat viruses belong to the species SARSr-CoV according to the criteria for species demarcation in the subfamily



Fig. 1. A map of China illustrating the location of trap sites in which bats (red circles) were captured.

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