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SARS-CoV-2 host prediction based on virus-host genetic features

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ABSTRACT

The genetic diversity of the Coronaviruses gives them different biological abilities, such as infect different cells and/or organisms, a wide spectrum of clinical manifestations in a single host, their different routes of dispersion, and viral transmission in a specific host. In recent decades, different Coronaviruses have emerged that are highly adapted for humans and causing serious diseases, leaving their host of unknown origin. The viral genome information is particularly important to enable the recognition of patterns linked to their biological characteristics, such as the specificity in the host-parasite relationship. Here, based on a previously computational tool, the seq2host, we developed a novel approach which uses new variables obtained from the frequency of spike-Coronaviruses codons, the Relative Synonymous Codon Usage (RSCU) to shed new light on the molecular mechanisms involved in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) host specificity. By using the RSCU obtained from nucleotide sequences before the SARS-CoV-2 pandemic, we assessed the possibility of know the hosts capable to be infected by these new emerging species, which was first identified infecting humans during 2019 in Wuhan, China. According to the tool trained and validated using sequences available before the pandemic, bats are the most likely susceptible host to the SARS-CoV-2 infection, as previously suggested in other studies that searched for the host viral origin.

Introduction

The first reported case of Coronavirus Disease 2019 (COVID-19) occurred in the city of Wuhan, Hubei province, China, in late December 2019, although retrospective analyses identified a patient with symptoms in early December^{1,2}. The viral agent associated with this new pneumonia - The Severe Acute Respiratory Syndrome associated with Coronavirus 2 (SARS-CoV-2) (*Sarbecovirus* subgenus, *Betacoronavirus* genus) - quickly spread worldwide causing a pandemic with global impact³. SARS-CoV-2 represents the seventh know coronavirus to circulate in a human-to-human transmission chain of the *Coronaviridae* family. While the SARS-CoV, MERS-CoV, and SARS-CoV-2 can cause severe disease, the HKU1, NL63, OC43, and 229E cause mild symptoms, and for all of them, their ancestral hosts are not human⁴.

Viruses related to SARS-CoV-2 genome have been found in bats⁵ and pangolins (*Manis javanica*)^{6,7} in the Asian continent. The divergence of the SARS-CoV-2 and the related viruses represents decades of evolution⁸, which makes it difficult to suggest which is the probable host involved in the SARS-CoV-2 emergence. Although the biological mechanism responsible for viruses spillover remains uncertain^{9,10}, since its first description, this virus has shown sustained transmission in the human-to-human transmission chain without adaptive genomic changes¹¹, demonstrating its pandemic potential.

The Coronaviruses are a group of viruses that differ from each other by their genomic characteristics and their ability to infect different groups of organisms¹². They are members of the subfamily *Coronavirinae* in the family *Coronaviridae* and the order *Nidovirales* (International Committee on Taxonomy of Viruses). Studies have shown that these viruses can have abnormally high replication fidelity¹³, having in their genomic structure, a set of RNA processing enzymes that have improved the low fidelity of RNA replication¹⁴. Despite having important genomic conservation at apical levels of their phylogenetic structure, they present considerable genetic differences at basal levels, this difference being important for studies that explore levels of adaptation to different hosts¹². The discriminative characteristics that make it possible to distinguish SARS-CoV-2 from other related coronaviruses (SC2r-CoVs) to it are concentrated mainly in the Spike gene, which appears to be the main component of the virus associated with host specificity¹⁵.

Genomic features of the SARS-CoV-2 spike protein structure are known to be linked to its ability to infect humans¹⁶. Sequences coding to the Spike protein were previously used to build a computational tool for inference of potential hosts using genomic data¹², such as mononucleotide and dinucleotide composition. Here, we applied a new approach using the Relative Synonymous Codon Usage (RSCU) to training and validate the seq2host tool developed by Tang *et al*¹² and we complemented this previous study using the recent information and machine learning techniques as Principal component analysis (PCA) and

Mahalanobis distance (MD). For this purpose, we use this classifier to find the MD of new coronaviruses sequences and host prototypes, suggesting other possible hosts.

Results

Phylogenetic analysis, dimensionality reduction, and model training

The phylogenetic analysis based in the aligned aminoacid sequences indicated that the sequences are grouped into four large groups, characterized as Coronaviruses genera, in the *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus*, and *Gammacoronavirus* (Figure 1A). The reduction of the 59 features (obtained from the genome through RSCU codification) into two-dimension space with Principal Component Analysis (PCA) enables us to visualize a scatter plot of the first two dimensions of Dataset 1 (Figure 1B-C) containing all samples of each host together with their prototypes (centroids). In general, the points of some viral species, such as bovine and murine (blue and pink colors, respectively) concentrates in one specific point cloud, indicating that contains relevant information about the host specificity, but in other cases, such as to the human and bats, the points are spaced in space.

Validation

The validation of rating performance was obtained using the Dataset-1 and indicated a progressive increase in accuracy with an increase in the number of components retrieved by the PCA calculation (Figure 2). With 2 components the total explained variance obtained was 0.52593 with accuracy of prediction 0.668493 whereas with 20 components variance and accuracy increased to 0.9683 and 0.993151 respectively so the prediction of the sequences from the remaining datasets was computed with the 20-dimensional projection distances measures.

Host predictions for viruses suspected of involving transmission between species

The trained model was applied to 47 additional sequences (Dataset-2) that we only listed the hosts from which they were isolated, with all the samples with evidence of potential transmission between species, as used previously¹². To the sequences isolated from palm civets, here called Civets-CoVs (SARS-CoV), the predicted host was humans. According to Tang¹², bats and humans are the hosts of these viral strains, but bats are the preferable hosts; the porcine-CoV, was a SARS-associated coronavirus that was transmitted from human to porcine, and the prediction results indicated the same results obtained to the dromedary-CoVs, with bats as the predicted host; the SARS-like coronaviruses sequences isolated from bats, the results obtained using MD correctly confirmed the first host as bats, followed by humans; to the sequences isolated from Dromedary, the Dromedary-CoVs (MERS-CoV), obtained after the outbreak in the Middle East in 2012 indicated that humans and bats are the first and second host, respectively and is corroborated by the first study¹². The MD distances to the Coronavirus sample isolated from Alpaca (Alpaca-CoVs) indicated that the predicted host was bovine; surprisingly, the to the bovine-CoV (Human enteric coronavirus isolated from bovine) result, the predicted host was bovine; and to the Human-CoV (Human enteric coronavirus isolated from human) sequence, the predicted host was bovine (Table 1).

SARS-CoV-2 host prediction

To predict the SARS-CoV-2 host-associated, we used the classifier constructed and validated using MD and the Spike nucleotide sequences of the SARS-CoV-2 obtained from GenBank (Dataset-3). The predicted host was bat although samples were taken from humans (Table 1). The phylogenetically nearest sequences to SARS-CoV-2 (SC2r-CoV), Dataset-4 were predicted to be hosted by bats. This result is completely congruent with the scientific evidence raised so far, which found SC2r-CoVs viruses in bats. The other tested hosts were not involved in this hosting system according to the prediction. Figure 3 presents all datasets in a two dimensional PCA scatter plot.

Discussion

Since the beginning of the pandemic, the origin of SARS-CoV-2 has been an object of interest to the global scientific community, with several scientific initiatives carried out and even, with the World Health Organization (WHO) moving a mission as part of the One Health approach, to identify the zoonotic source of the virus and routes of introduction to the human population in China, including the possible role of intermediate hosts. Genomics comparisons of SC2r-CoVs and pandemic SARS-CoV-2 sequences suggested that the virus has required little to no significant adaptation to start a circulation in humans at the early phase of the pandemic¹¹. The structural changes present in the SARS-CoV-2 when compared to SC2r-CoVs, which may be associated with a high capacity to infect human cells, are in the most divergent genome region, that coded to the Spike protein¹⁶. Two of the key changes that occur in the virus are the specific receptor binding domain sequence and the inserted furin cleavage site^{8,16}. Molecular evidence have shown that the coronaviruses most highly related to the SARS-CoV-2 are found in bats and pangolins⁵⁻⁷, suggesting that these mammals may be the reservoir of the virus that causes COVID-19.

Throughout the natural course of coronavirus evolution, virus genomes accumulate mutations during their propagation. The underlying principle is that virus-host genetic information can provide the variables involved in the host infection specificity process, a valuable tool to explore the taxonomic classification of new viruses, track transmission chains, and the prediction of hosts that are likely to be involved in their replication cycles¹². Recently, an approach using dual statistical models based on mono- and dinucleotide composition was used to predict the probable hosts for viruses belonging to the genus *Coronavirus*¹². The results retrieved using a new group of sequences, the SARS-CoV-2 spike gene sequences (Dataset-3) as input for host prediction using the previous approach (Seq2Host platform)¹², indicated that organisms predicted as hosts are avian and bovine for first and second prediction using the Mahalanobis Distance technique. The current evidence suggests that this result is not consistent with studies exploring evolutionary and biological aspects related to the virus, which show that both groups of organisms are not involved in the zoonotic circulation of SARS-CoV-2-related viruses, as well as SARS-CoV-2 has not yet been found in these organisms. The hosts known to be involved in viral circulation are bats (in a zoonotic environment) and humans, although there is still a discussion about the intermediate host in this emergence process. To explore the same approach, but now refining it to be precise in identifying hosts with a description known and sustained in the literature, we use a new way to recover features associated with virus-host specificity, the RSCU signature.

Our results indicated that the main group of organisms that are among those tested (avian, bat, bovine, human, murine, and porcine) that are involved as natural hosts for SARS-CoV-2 are bats based in the MD of the SARS-CoV-2 sequences to the prototypes. These findings suggested that the RSCU can reflect biological meaning in terms of coronavirus adaptation to the cellular host machinery. According to a recent modeling study, the SARS-CoV-2 Spike protein could have a high probability of binding well with the ACE2 receptors in rats, sheep, camels, and squirrels¹⁷. Recently, the joint international team WHO-China study concluded that the most closely related genomic sequences to the SARS-CoV-2 have been found in bats¹⁸.

Beyond the prediction results obtained using the RSCU calculations based in the MD, our study suggests that SARS-CoV-2 emerged to humans from bats. Furthermore, the high susceptibility and permissivity of mink and cats to the SARS-CoV-2 suggest that additional species of animals may act as a potential reservoir to the virus^{19,20}. This new approach can be used to the: (i) description of the natural host, and the viral emergency host (viral spillover) in new *Coronavirus* emergence; (ii) It may be important to suggest new experimental models that are biologically close to natural hosts; (iii) It can be useful to study new coronaviruses with emergency potentials.

Methods

Datasets

To conduct this study, we based on the datasets previously described¹². First, to the training tool, we used the same dataset used in the work previously developed¹², with 730 sequences corresponding to the Spike gene of different viral specimens of coronavirus (Dataset-1), 196 of which belong to the human host, 182 to the porcine, 173 to the avian, 77 to the bovine, 74 to the bat and 28 to the murine. To test the model using sequences that are not in the training data, we also used the same dataset provided previously by the authors as a test set (Dataset-2) corresponding to 47 spike gene sequences of SARS coronavirus - 30 collected from civets, 1 from raccoon and 1 from porcine, 9 Middle East respiratory syndrome coronavirus from dromedary, 3 Bat SARS-like coronavirus from bats, 1 Bovine coronavirus isolated from Alpaca and 2 Human enteric coronavirus - 1 from human and 1 from bovine.¹² To apply the model to the prediction of the viral hosts involved in the viral replication biology of SARS-CoV-2, we used 86 virus sequences retrieved from GenBank at 04-26-2020, all of them isolated from human samples (Dataset-3).

Phylogenetic Analysis

The phylogenetic inference was performed with a previously curated dataset (dataset-1)¹². A phylogenetic tree was reconstructed based on Spike gene using the Maximum Likelihood (ML) method implemented in IQ-TREE 1.5.5²¹ with automatic model selection by ModelFinder and using the Bayesian Information Criterion (BIC)²². The robustness of the groupings observed was assessed using an ultrafast bootstrap approximation (UFboot) during 1,000 replicates. The ML tree was visualized and plotted using FigTree v.1.4.3²³. Taxon labels for sequences used in this work had the format: accession number/Coronavirus genera/Primary host.

RSCU calculation

The codon is a combination of three nucleotides that encodes for an amino acid or a stop signal. Despite of there are 64 combinations of three nucleotides, considering that there are four nucleotides for the coding purpose, the number of amino acids are 20, because some amino acids can be coded by more than one triplet of nucleotide. So the genetic code is redundant, and it is described “degenerate”, that is, multiple synonymous codons refer to the same amino acid. For example, the codons GCU, GCC, GCA and GCG code the same amino acid Alanine (*Ala*).

The usage of synonymous codon for each amino acid is not random, it depends on the abundance of the respective tRNA of the organism. Selective pressure contributes to optimize gene expression inducing a bias to the presence of codons related to more abundant tRNA species²⁴.

The expected number of occurrences of codon usage E_i for a given amino acid i can be computed counting the number of that codon in the sequence normalizing it by the number of codons that code the same amino acid:

$$E_i = \frac{1}{n_i} \sum_{j=1}^{n_i} F_j^{(i)},$$

where n_i is the number of synonymous codons for amino acid i ($1 \leq n_i \leq 6$) and $F_j^{(i)}$ is the number of occurrences of codon j for amino acid i . For example, for the amino acid Alanine (Ala), the number of synonymous codons is $n_{Ala} = 4$, whereas $F_{GCU}^{(Ala)}$, $F_{GCC}^{(Ala)}$, $F_{GCA}^{(Ala)}$, $F_{GCG}^{(Ala)}$ are the frequencies of its synonymous codons.

So, in the case of Alanine, its expected number of occurrences of codon usage is $E_{Ala} = \frac{1}{4} (F_{GCU}^{(Ala)} + F_{GCC}^{(Ala)} + F_{GCA}^{(Ala)} + F_{GCG}^{(Ala)})$.

The bias of each codon for a given amino acid that can be coded by more than one codon is estimated by calculating the Relative Synonymous Codon Usage $RSCU_j^{(i)}$ that is the number of times the codon j appears within a gene divided by the expected number of occurrences of its synonymous codons for the i^{th} amino acid:

$$RSCU_j^{(i)} = \frac{F_j^{(i)}}{E_i},$$

where $F_j^{(i)}$ is the number of occurrences of the j^{th} codon for the i^{th} amino acid, which is encoded by n_i synonymous codons²⁴. In the case of Alanine:

$$RSCU_{GCU}^{(Ala)} = \frac{F_{GCU}^{(Ala)}}{E_{Ala}} ; RSCU_{GCC}^{(Ala)} = \frac{F_{GCC}^{(Ala)}}{E_{Ala}} ; RSCU_{GCA}^{(Ala)} = \frac{F_{GCA}^{(Ala)}}{E_{Ala}} \text{ and } RSCU_{GCG}^{(Ala)} = \frac{F_{GCG}^{(Ala)}}{E_{Ala}}.$$

The values for UGG and AUG that codes respectively for Tryptophan and Methionine are always 1.0, since there is only one codon for them. In addition, the three stop codons can be excluded from the analysis, since they do not correspond to a tRNA. Computing the RSCU for valid codons for each sequence results in a 59 dimension feature space.

Viruses depend on the host machinery to replicate themselves and translate their proteins. As they require available tRNAs, they have the tendency to evolve a codon usage preference for the amino acid that can be coded by more than one codon, as closer as possible to their host. Therefore, the efficiency of the viral proteins production can be established selectively on genetic material mutation²⁵. In this way, we hope that the RSCU can identify how close the viral species are to each host specified in the training data set.

Dimensionality Reduction

Our model consists of using the nearest prototype classifier with Mahalanobis distance after the PCA reduction in the feature table with the RSCU values.

Principal components analysis (PCA) is a popular approach for deriving a low-dimensional set of features from a large set of variables. Its is widely used in Bioinformatics to analysis of genome data and gene expression levels²⁶.

The goal of PCA is to find the directions of maximum variance in high-dimensional data and projects (by a linear transformation) it onto a new subspace with equal or fewer dimensions than the original one. The orthogonal axes, called "principal components", of the new subspace can be interpreted as the directions of maximum variance given the constraint that the new feature axes are uncorrelated to each other²⁷.

In our case, we used PCA to reduce features dimensions from 59-dimension to smaller dimensional spaces starting with 2 components and increasing them. In order to visually explore the data, we used 2 components to plot the graphs presented in this text, Principal Component 1 (PC1) at the vertical axis x_1 and Principal Component 2 (PC2) at the horizontal axis x_2 .

The Nearest Prototype Classifier

In a supervised classification, the k -nearest neighbor (KNN), or even the nearest neighbor (when $k = 1$), can be used to classify an unknown sample, assuming that it will have characteristics similar to that of the neighborhood. The method consists of computing the distance to all the known samples and check the label of the k (usually an odd number to avoid ties) closest ones.

The unknown sample is labeled by the majority of votes from its k neighbours. In this study, instead of using the KNN classifier, we consider using the nearest prototype classifier.

In the training dataset, there are six labels (avian, bat, bovine, human, murine, and porcine), corresponding to the hosts of viral species, each one encompassing a certain amount of samples.

We define the prototype of each label as the mean point in the feature space of all species that infect the same host. For example, reducing the feature space to two dimensions x_1 and x_2 axis, we can find the prototype in the center of the points that belong to the same host computing the average for these features for each host:

$$\mathbf{c}_{\text{host}} = (\bar{x}_{1\text{host}}, \bar{x}_{2\text{host}}),$$

where $\bar{x}_{1\text{host}}$ is the average of x_1 values on the vertical axis and $\bar{x}_{2\text{host}}$ is the average of x_2 values on the horizontal axis for a given host. Note that $\mathbf{c}_{\text{host}}$ after the PCA reduction is a point with two coordinates $\mathbf{c}_{\text{host}} = (c_1, c_2)$, and there will be six prototypes in our training data set: $\mathbf{c}_{\text{avian}}$, $\mathbf{c}_{\text{bat}}$, $\mathbf{c}_{\text{bovine}}$, $\mathbf{c}_{\text{human}}$, $\mathbf{c}_{\text{murine}}$ and $\mathbf{c}_{\text{porcine}}$.

These prototypes with the mean of the values in each dimensional axis for the species that infect the same host are centroids and can be used to represent all the samples that belong to the corresponding host. So, instead of compute the distances for all the labeled points, the distance to the nearest prototype can be used to deduce the label of the unknown sample.

Let's say that D is the set of distances $d(\mathbf{x}, \mathbf{c}_{\text{host}})$ from each of the prototypes to the unknown point $\mathbf{x}_{\text{new}}$ with $\mathbf{x}_{\text{new}} = (x_1, x_2)$:

$$D = \{d(\mathbf{x}, \mathbf{c}_{\text{avian}}), d(\mathbf{x}, \mathbf{c}_{\text{bat}}), d(\mathbf{x}, \mathbf{c}_{\text{bovine}}), d(\mathbf{x}, \mathbf{c}_{\text{human}}), d(\mathbf{x}, \mathbf{c}_{\text{murine}}), d(\mathbf{x}, \mathbf{c}_{\text{porcine}})\}.$$

We can assume that the label of the unknown point will be the label of the shortest distance in this set:

$$\text{host}(x) = \text{host}(\min D).$$

Modeling, validation and prediction

Since the unknown host sample prediction depends on the distance computation to prototypes, we need to define which metric (distance measure) is more appropriate for our classification problem. In fact, in our case, we are interested in computing the distance between a z -dimensional point $\mathbf{x} = (x_1, x_2, \dots, x_z)$ and the centroid $\mathbf{c} = (c_1, c_2, \dots, c_z)$ of a cloud of points representing a given host. Indeed, this distance can be measured by several methods. The most known is the Euclidean distance given by $d_{\text{ED}}(\mathbf{x}, \mathbf{c}) = \sqrt{(\mathbf{x} - \mathbf{c})(\mathbf{x} - \mathbf{c})^T} = \sqrt{\sum_{i=1}^n (x_i - c_i)^2}$. But there are other types of distance measures that can reflect more precisely the distribution of the host points in the dataset. One of them is the Mahalanobis distance (MD)²⁸⁻³⁰ which is a measure between a sample point $\mathbf{x} = (x_1, x_2, \dots, x_z)$ and a distribution of sample points represented by:

- (i) its centroid $\mathbf{c} = (c_1, c_2, \dots, c_z)$ and
- (ii) its covariance matrix S .

The distance d_{MD} is given by $d_{\text{MD}}(\mathbf{x}, \mathbf{c}) = \sqrt{(\mathbf{x} - \mathbf{c})S^{-1}(\mathbf{x} - \mathbf{c})^T}$.

Taking as an example of our analysis, we will have 74 samples extracted from **bats** and the corresponding 2-dimensional points after the PCA reduction. The centroid of bat will be $\mathbf{c}_{\text{bat}} = (c_{1\text{bat}}, c_{2\text{bat}}) = (\bar{x}_{1\text{bat}}, \bar{x}_{2\text{bat}})$, and the covariance matrix S_{bat} for the points that belong to bat will be:

$$S_{\text{bat}} = \begin{bmatrix} \sigma_{x_{1\text{bat}}}^2 & \sigma_{x_{1\text{bat}}} \sigma_{x_{2\text{bat}}} \\ \sigma_{x_{1\text{bat}}} \sigma_{x_{2\text{bat}}} & \sigma_{x_{2\text{bat}}}^2 \end{bmatrix},$$

where:

- $\sigma_{x_{1\text{bat}}}$ is the variance on vertical axis of the points that belong to the host bat and
- $\sigma_{x_{2\text{bat}}}$ is the variance of these points on horizontal axis.

MD is the distance between a point and a set of points in a multivariate space. If the data are non correlated MD it would be as Euclidean distance. It measures distance relative to the centroid, which is the prototype in our case.

MD can improve the accuracy of estimates because it considers correlations between the summary statistics³⁰. The use of MD after use PCA exhibit some advantages in terms of computational cost as in the calculation of the covariance matrix S and its inverse S^{-1} .

The validation is the performance evaluation of the model. This was done with the technique leave-one-out cross-validation in the training dataset (Dataset-1). It consists in removing one point at a time from the dataset to be the unknown point to be

predicted and calculating its distance to the grouped points generated according to the host label of the remaining sequences. The shortest distance obtained is considered the prediction and compared to the known label of the point. If there is a match, it is added to the count to calculate the accuracy. This procedure is repeated to all the sequences of the training dataset. In the end, the relative accuracy can be calculated counting the hits in the prediction. The validation was carried out for several dimensions starting from the reduction of characteristics to two dimensions and increasing until the accuracy reached a level in which there were no relevant changes, which occurred in 20-dimension.

All the calculations were made in Python 3³¹ programming language with Numpy³² library. Graphics made with Matplotlib³³ library.

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Author contributions statement

I.Y.K., M.P.C., and R.F.H. conceived the experiments; I.Y.K., M.P.C., and R.F.H. conducted the experiments; and all authors analysed the results. All authors wrote the original draft and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

To include, in this order: **Accession codes** (where applicable); **Competing interests** (mandatory statement).

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Figures

Figure 1. Characterization of the training dataset (Dataset 1). (A) Phylogenetic characterization showing all the Coronaviruses genus; (B) PCA reduction with prototypes according to the different genus; and (C) PCA reduction with prototypes according to the different primary host. The different colours represent each group of genus or host class in the training data set (Dataset 1).

Figure 2. Classifier performance. (A) Cumulative explained variance using distinct number of principal components; and (B) Accuracy to each host using different number of principal components.

Figure 3. PCA reduction, all datasets. Dataset-1, training dataset; Dataset-2, testing; Dataset-3, SARS-CoV-2; Dataset-4, Bat Coronavirus, HCoV and Pangolin Coronavirus.

Number of sequences	Isolation source	Natural host	Predicted host	Coronavirus specie	Dataset
30	civet	human	human	SARS-CoV-1	2
1	raccoon	human	human	SARS-CoV-1	2
9	dromedary	human	human	MERS-CoV	2
1	porcine	human	human	SARS-CoV-1	2
3	bat	bat	bat	Bat SARS-like	2
1	alpaca	bovine	bovine	Bovine Coronavirus	2
1	human	human	bovine	Human enteric coronavirus	2
1	bovine	human	bovine	Human enteric coronavirus	2
86	human	human	bat	SARS-CoV-2	3
5	bats or pangolin	bat	bat	SC2r-CoVs	4

Table 1. Sequences used to the biological validation and prediction results for Datasets 2, 3 and 4.