IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"FEATURES OF CORONAVIRUSES: SARS-COV, MERS-COV & SARS-COV-2 (2019-nCOV) BRIEF REVIEW"

Bhosale Sarika

Bhosale Sarika, Department of Microbiology, Bharati Vidyapeeth deemed to be University, Yashwantrao Mohite College of Arts, Science and Commerce, Pune - 411038, India

Abstract

Members of the family Coronaviridae cause a broad spectrum of animal and human diseases. Until 2003, coronaviruses attracted little interest beyond causing mild upper respiratory tract infections. This changed dramatically in 2003 with the zoonotic SARS-CoV (*Severe Acute Respiratory Syndrome Coronavirus*) and the more recent emergence of MERS-CoV (*Middle East respiratory syndrome Coronavirus*) has confirmed the coronaviruses as significant causes of severe respiratory disease. SARS-CoV is thought to be an animal virus from an as-yet-uncertain animal reservoir, perhaps bats, that spread to other animals (civet cats) and first infected humans in the Guangdong province of southern China in 2002(WHO 2020) and spread to five continents through air travel routes, infecting 8,098 people and causing 774 deaths. Since 2012, MERS-CoV has been emerged in the Arabian Peninsula and was exported to 27 countries including Algeria, Austria, Bahrain, China, Egypt, France, Germany, Greece, Islamic Republic of Iran, Italy, Jordan, Kuwait, Lebanon, Malaysia, the Netherlands, Oman, Philippines, Qatar, Republic of Korea, Kingdom of Saudi Arabia, Thailand, Tunisia, Turkey, United Arab Emirates, United Kingdom, United States, and Yemen (WHO 2019). Total of ~2,494 individuals and claiming 858 lives were reported due to the infection of MERS-CoV. SARS-CoV-2 is associated with an on-going outbreak of atypical pneumonia (Covid-2019) that has affected over 209 countries and territories around the world and two international conveyances the *Diamond Princess* Cruise ship harboured in Yokohama, Japan, and the Holland America's *MS Zaandam* cruise ship. Up to 14, 46, 981 people affected of which 1, 055,207 active cases and 83,090 deaths are declared 08 April 2020. On January 30th 2020, the World Health Organization declared the SARS-CoV-2 epidemic as a public health emergency of international concern.

Keywords : : *SARS-CoV*, *MERS-CoV* , *SARS-CoV-2* (*2019-nCoV*), Coronavirus diseases

INTRODUCTION

In a timeline that reaches the present day, an epidemic of cases with unexplained low respiratory infections detected in Wuhan, the largest metropolitan area in China's Hubei province, was first reported to the WHO Country Office in China, on December 31, 2019. Published literature can trace the beginning of symptomatic individuals back to the beginning of December 2019. As they were unable to identify the causative agent, these first cases were classified as "pneumonia of unknown etiology." The Chinese Center for Disease Control and Prevention (CDC) and local CDCs organized an intensive outbreak investigation program. The etiology of this illness is now attributed to a novel virus belonging to the coronavirus (CoV) family.

Coronaviruses possess a distinctive (idiosyncratic) morphology, the name being derived from the outer fringe, or “corona” of embedded envelope protein. Since the first reports of novel pneumonia "COVID-19," this is the acronym of “coronavirus disease 2019” or *SARS-CoV-2* in Wuhan, Hubei province, China 1,2, there has been considerable discussion on the origin of the causative virus, *SARS-CoV-2* 3 (also referred to as HCoV-19) 4. Since 2005, dozens of different coronaviruses have been isolated from bats, which indicate that human respiratory coronaviruses, *SARS coronavirus*, and *MERS coronavirus*, may each have originally emerged from ancestral bat viruses.

TAXONOMY

Coronaviruses and *Toro viruses* are two virus genera within the virus family Coronaviridae. The family Coronaviridae, Arteriviridae and Roniviridae are three RNA virus families within the order Nidovirales. Later two families contain pathogens of birds and insects, respectively. The family Coronaviridae consists of two subfamilies, Coronavirinae and Torovirinae, the latter containing viruses causing mainly enteric infections of horses, cattle, pigs, cats, and goats.. Members of the Torovirinae subfamily are not as yet known to cause human infections. Members of the subfamily Coronavirinae are subdivided into four genera. The genus *Alphacoronavirus* contains the human virus *HCoV-229E*, one other human coronavirus (*HCoV-NL63*), and many animal viruses. The genus *Betacoronavirus* includes the prototype *Mouse hepatitis virus* (MHV), the three human viruses *HCoV-OC43*, *SARS-HCoV*, and *HCoV-HKU1*, and the SARS-related coronavirus, *Middle Eastern respiratory syndrome* (MERS) *coronavirus*, together with a number of animal coronaviruses. The genus *Gammacoronavirus* contains viruses of cetaceans (whales) and birds, and the genus *Deltacoronavirus* contains viruses isolated from pigs and birds.

Properties of Coronaviruses

1. Virion is pleomorphic spherical 80 to 220 nm (*Coronaviruses*); or disc, kidney, or rod shaped 120 to 140 nm (*Toro viruses*).

2. Envelope with large, widely spaced club-shaped peplomers.

3. Tubular nucleocapsid with helical symmetry.

4. Linear, plus sense single stranded RNA genome 27 to 33 kb, capped, polyadenylated, infectious, untranslated sequences at each end.

5. Three or four structural proteins: nucleoprotein (N), peplomer glycoprotein (S), transmembrane glycoprotein (M), sometimes hemagglutinin-esterase (HE).

6. Genome encodes 3 to 10 further non-structural proteins, including the RNA-dependent RNA polymerase made up of subunits cleaved from two polyproteins translated from the 5′-end.

7. Replicates in cytoplasm; genome is transcribed to full-length negative sense RNA, from which is transcribed a 3′-coterminal nested set of mRNAs, only the unique 5′ sequences of which are translated.

8. Virions are assembled and bud into the endoplasmic reticulum and golgi cisternae; release is by exocytosis.

9. Variant viruses arise readily, by mutation and recombination, and the use of different receptors influences the host range exhibited.

Coronaviruses are well-established pathogens of humans and animals while the *Toro viruses* are recognized as causes of animal diarrhoea. All coronaviruses share a common pleomorphic morphology and possess positive sense single stranded RNA genome ~30 000 nucleotides (up to 30 kb) long, 80 × 160 nm diameter, with 12–24 nm surface projections (spikes) that cause the corona (Latin: crown) appearance. Coronaviruses are classified into three groups, initially based on antigenic relationships of the spike (S), envelope (E) membrane (M) and nucleocapsid (N) proteins and now re-enforced by viral genetic phylogeny. Uniquely, replication of the RNA genome proceeds through the generation of a nested set of viral mRNA molecules. It is thought that human coronaviruses enter host cells, predominantly, by specific receptors. Aminopeptidase-N and a sialic acid-containing receptor have been identified to act in such a role for *229E* and OC43 respectively. After the virus enters the host cell and uncoats, the genome is transcribed and then translated. A unique feature of replication is that all the mRNAs form a "nested set" with common 3′ ends; only the unique portions of the 5′ ends are translated. There are 7 mRNAs produced. The shortest mRNA codes for the nucleoprotein and the others each direct the synthesis of a further segment of the genome. The proteins are assembled at the cell membrane and genomic RNA is incorporated as the mature particle forms by budding from internal cell membranes.

In 1965, Tyrrell and Bynoe 5 cultured a virus obtained from the respiratory tract of a boy with a common cold by passage in human embryonic tracheal organ cultures. The media from these cultures consistently produced colds in volunteers. The agent was ether sensitive but not related to any known human virus. Subsequently, electron microscopy of fluids from infected organ cultures revealed particles that resembled infectious bronchitis virus of chickens.10 At about the same time, Hamre and Procknow recovered a cytopathic agent in tissue culture from medical students with colds.11 The prototype virus was named *229E* and was found on electron microscopy to have a similar or identical morphology (Fig.1).The *HCoVs 229E* and *NL63* are group 1 coronaviruses, while *OC43, HKU-1* and *SARS* coronaviruses are classified in group 2. Group 3 coronaviruses are found in avian species. Genetic recombination freely occurs between members of the same and of different coronavirus groups providing chances for increased genetic diversity. Efforts to identify the animal reservoir of SARS coronavirus led to the discovery of diverse bat coronaviruses in both group 1 and 2 that are closely related phylogenetically to different mammalian coronaviruses. It has been proposed that bat coronaviruses may certainly have been the ancestors of many mammalian coronaviruses. The recent studies on the comparative evolution of animal and human coronaviruses have led to the conclusion that *HCoV 229E* and *OC43*, which causes common cold, are now universally endemic in humans. Crossed species from their animal reservoirs mainly bats and cattle to humans within the last 200 years, demonstrates the fact that coronaviruses continuously

cross species barriers and causes novel diseases. Some coronaviruses that infect animals have become able to infect humans and then spread between people, but this is rare. Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) are examples of diseases caused by coronaviruses that originated in animals and spread to people. This is what is suspected to have happened with the virus that caused the current outbreak of COVID-19 in Central China – Wuhan. However no one knows the exact source of this virus. Public health officials and companions are working hard to ascertain the source of COVID-19. Huanan Seafood Wholesale Market, where the Coronavirus pandemic is believed to have started, was one of the largest marketplaces in Wuhan city with throngs of customers daily. The market was shut on January 1st 2020 after dozens of workers there had contracted the disease (Chinese Centre for Disease Control and Prevention) but now the virus is spreading from person to person. The coronavirus most similar to the virus causing COVID-19 is the one that causes SARS. Examples of some species of human corona viruses

1. Coronavirus Human coronavirus 229E
2. Coronavirus Human coronavirus OC43
3. Coronavirus Human coronavirus NL63
4. Coronavirus Human coronavirus HKU1
5. Coronavirus Severe acute respiratory syndrome coronavirus
6. Coronavirus Human enteric coronavirus

The current classification of coronaviruses includes taxa at eight out of the fifteen available ranks **3**, and it recognizes forty-nine species in twenty-seven subgenera, five genera and two subfamilies that belong to the family Coronaviridae, suborder Cornidovirineae, order Nidovirales, realm Riboviria **12**-**13**.14The family classification and taxa naming (taxonomy) are developed by the Coronavirus Study Group (CSG), a working group of the International Committee on Taxonomy of Viruses (ICTV) **15**.(Fig 1)

Figure 1. Phylogeny of Coronaviruses

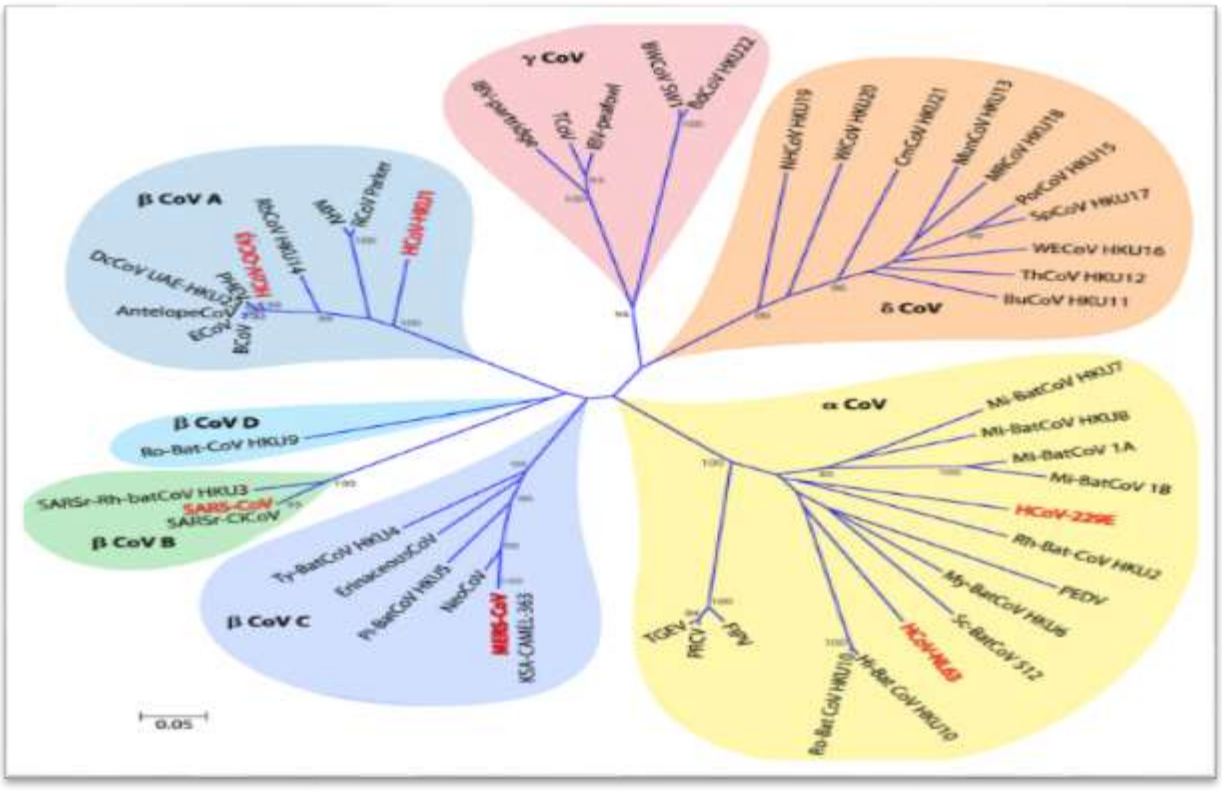
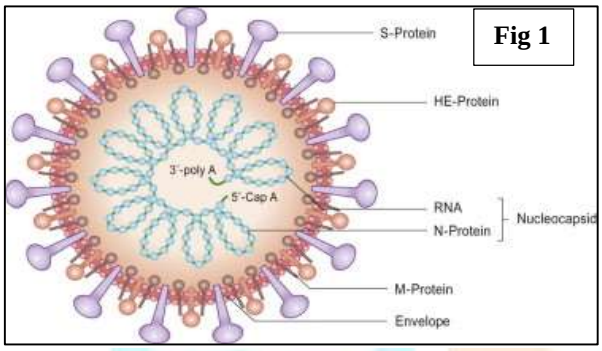
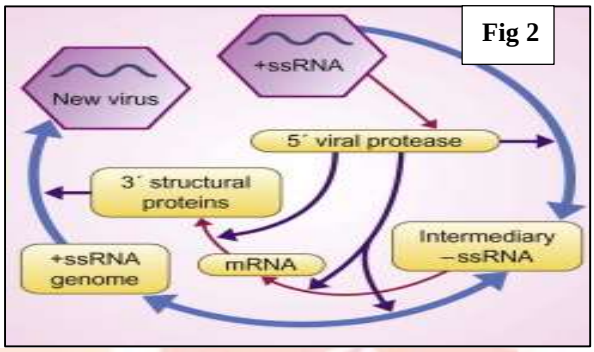
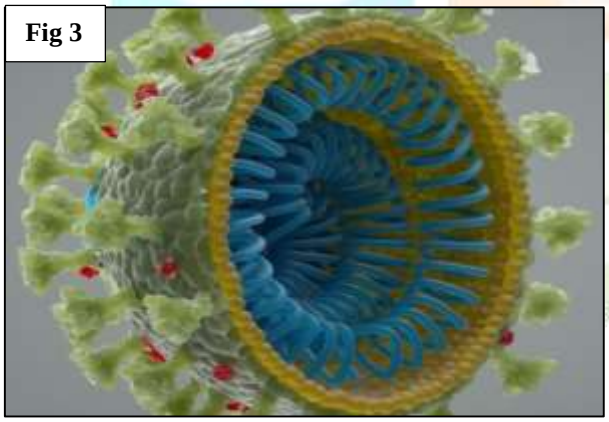


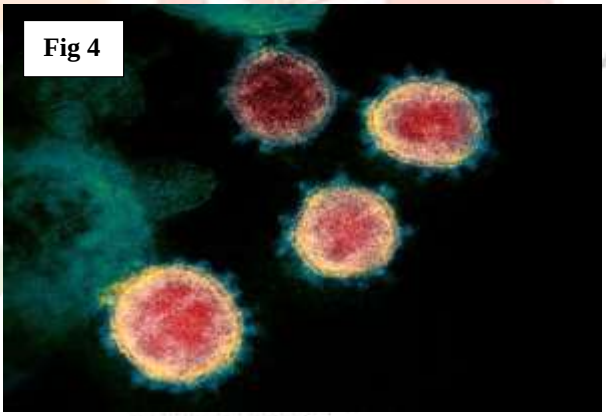
Fig 1. Phylogenetic tree of 50 coronaviruses constructed by the neighbour-joining method using MEGA 5.0 using partial nucleotide sequences of RNA-dependent RNA polymerase. The scale bar indicates the estimated number of substitutions per 20 nucleotides. Space does not permit providing full virus names, except for the human viruses, which are scattered among the viruses isolated from many other species (major pathogens shown in red): *HCoV-229E*, *human coronavirus 229E*; *HCoV-HKU1*, *human coronavirus HKU1*; *HCoV-NL63*, *human coronavirus NL63*; *HCoV-OC43*, *human coronavirus OC43*; *KSA-CAMEL-363*, *KSA-CAMEL-363* isolate of Middle East respiratory syndrome coronavirus; *MERS-CoV*, Middle East respiratory syndrome coronavirus; *MHV*, murine hepatitis virus, the prototypic virus of the family; *SARS-CoV*, SARS coronavirus; *SARSr-CiCoV*, SARS-related palm civet coronavirus. A remarkable number of the viruses represented here are from bats, many different species of bats, and quite a few of these are rather closely related to *SARS-CoV*.

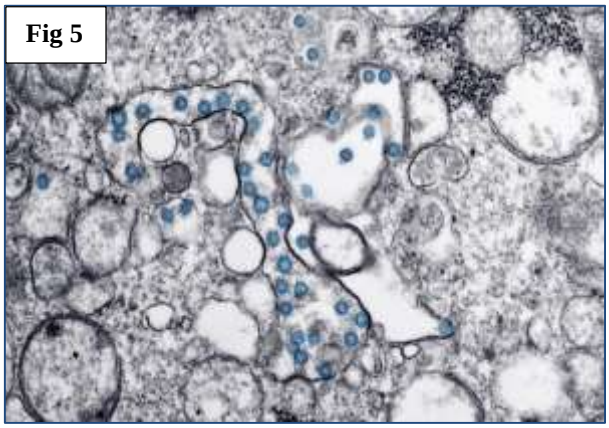
Figure 2 Coronavirus Important Pictures











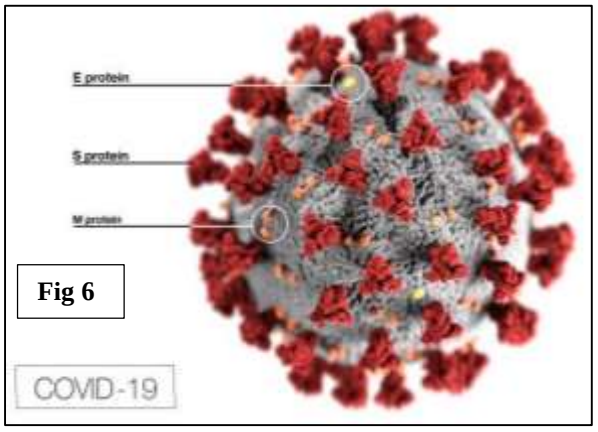


Fig 1. General structure of Coronavirus

Fig 2. Replication cycle of coronaviruses

Fig 3. Viruses like the novel coronavirus are shells holding genetic material. (Image: © Andriy Onufriyenko/Getty Images)

Fig 4. Researchers have identified microscopic features that could make the pathogen more infectious than the SARS virus — and serve as drug targets. An image of the new coronavirus taken with an electron microscope. (Source: U.S. National Institutes of Health/AP/Shutterstock)

Fig 5. Transmission electron microscopic image of an isolate from the first U.S. case of COVID-19, formerly known as *2019-nCoV*; the spherical viral particles, colorized blue, contain cross-sections through the viral genome, seen as black dots. Coronavirus, COVID-19. Contributed from the CDC, Hannah A Bullock; Azaibi Tamin (Public Domain)

Fig 6 Illustration of ultra-structural morphology exhibited by coronaviruses created at the Canters for Disease Control and Prevention (CDC), the spikes that adorn the outer surface of the virus, which impart the look of a corona surrounding the virion, when viewed electron microscopically. In this view, the protein particles E, S, and M, also located on the outer surface of the particle, have all been labelled as well. A novel coronavirus, named *Severe Acute Respiratory Syndrome coronavirus 2* (*SARS-CoV-2*), was identified as the cause of an outbreak of respiratory illness first detected in Wuhan, China in 2019. The illness caused by this virus has been named *coronavirus disease 2019* (*COVID-19*). Contributed from the CDC, Alissa Eckert, MS; Dan Higgins, MAM (Public Domain)

Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses, which is in authority for developing the certified classification of viruses and taxa nomenclature (taxonomy) of the Coronaviridae family, assessed the novelty of the human pathogen tentatively named *2019-nCoV* (n-novel). Based on phylogeny, taxonomy and conventional practice, the CSG formally differentiates this virus as a sister to *severe acute respiratory syndrome coronaviruses* (*SARS-CoVs*) of the species *Severe acute respiratory syndrome-related coronavirus* and labels it as *severe acute respiratory syndrome coronavirus 2* (*SARS-CoV-2*). *SARS-CoV-2* is the seventh coronavirus known to infect humans; *SARS-CoV*, *MERS-CoV* and *SARS-CoV-2* can cause severe disease, whereas *HKU1, NL63, OC43* and *229E* are associated with mild symptoms 16. To simplify communication, the CSG further recommends using the following naming convention for individual isolates: *SARS-CoV-2/Isolate/Host/Date/Location*. First and principal questions in relation to human coronavirus causing an outbreak of a respiratory syndrome that emerged in Asia in December 2019 novel? The questions of virus novelty and naming are now posed because virus first detected in Wuhan, China, was temporally named *2019 novel coronavirus, 2019-nCoV*. The term "novel" may refer to the disease (or spectrum of clinical manifestations) that is caused in humans infected by this particular virus, which, however, is only emerging and requires further studies 17,18. The term "novel" in the name of *2019-nCoV* may also refer to an incomplete match between the genomes of this and other (previously known) coronaviruses, if the latter was considered an appropriate criterion for defining "novelty". However, virologists agree that neither the disease nor the host range can be used to reliably ascertain virus novelty (or identity), since few genome changes may attenuate a deadly virus or cause a host switch 19. This question is answered in best practice by evaluating the degree of relatedness of the candidate virus to previously known viruses of the same host or established monophyletic groups of viruses, often known as genotypes or clades, which may or may not include viruses of different hosts.

During the 21st century, researchers studying coronaviruses – a family of enveloped positive-stranded RNA viruses of vertebrates 20 were challenged several times with the question of coronavirus novelty, including two times when a severe or even life-threatening disease was introduced into humans from a zoonotic reservoir: this happened with severe acute respiratory syndrome (SARS) 21, 22, 23, 24 and, a few years later, with Middle East respiratory syndrome (MERS) 25,26. Each time, the pathogen was initially called a new human coronavirus, as was the case with *SARS-CoV-2* during the current outbreak, every time the issue was resolved by the sequence-based family classification

To escalate the difference between severe acute respiratory syndrome-related coronavirus and *SARS-CoV*, i.e. between species and virus, it may be instructive to look at their relation in the context of the full taxonomy structure of several coronaviruses and in comparison with the taxonomy of the virus host, specifically humans (table 1). The current classification of coronaviruses includes taxa at eight out of the fifteen available ranks, 3 and it recognizes forty-nine species in twenty-seven subgenera, five genera and two subfamilies that belong to the family Coronaviridae, suborder Cornidovirineae, order Nidovirales, realm Riboviria 12 13 14 15 shown in following table (1) a comparison of the taxonomies of selected coronaviruses and the founders of virology. The CSG uses a computational framework of comparative genomics 27 that is shared by several study groups concerned with the classification and nomenclature of the order Nidovirales and coordinated by the Nidovirales Study Group 28.

Table (1): Taxonomies of Coronaviruses and Humans

Category	Virus	Host
Realm	Riboviria	
Order	Nidovirales	Primates
Suborder	Cornidovirineae	
Family	Coronaviridae	Hominidae
Subfamily	Coronavirinae	Homininae
Genus	Betacoronavirus	Homo
Subgenus	Sarbecovirus	
Species	Severe acute respiratory syndrome- related coronavirus	Homo sapiens
Individuum	SARS-CoV SARS-CoV_PC4-227 SARSr-CoV_BtKY72 SARS-CoV2/X1/Human/2019/Wuhan_XYZ12345	Dmitri Ivanovsky Martinus Beijerinck Friedrich Loeffler Paul Frosch

Table 1: The species and its viruses – a statement of the Coronavirus Study Group (CSG),
(Source: Alexander E. Gorbalenya, 2020 *Severe acute respiratory syndrome-related coronavirus*)

Thus, *SARS-CoV-Urbani* ((named after Dr. Carlo Urbani who was the first WHO officer to identify the outbreak of *SARS-CoV* disease in an American businessman who had been admitted to a hospital in Hanoi) with a particular genome sequence **29** could be regarded as equivalent to a single human being, while the species *Severe acute respiratory syndrome-related coronavirus* would be on a par with the species *Homo sapiens*. This parallel could go beyond semantics and be biologically meaningful because of how coronaviruses are assigned to species in practice, although the extension of this concept to virology is yet to be developed and thoroughly tested **30.**

In the past, the classification of coronaviruses was largely based on serologic cross reactivity involving the S protein till it became based on comparative sequence analysis of replicative proteins. The choice of proteins and the methods used to analyze them have gradually evolved since the start of this century **15**,**31**,**32**,**33**. Currently, the CSG analyzes 3CLpro, NiRAN, RdRp, ZBD and HEL domains **34** (**Fig. 2A**), which replaced the seven domains used for analysis between 2009 and 2015**13**. The Study Groups quantify and partition the variation in the most conserved replicative proteins encoded in open reading frames 1a and 1b (ORF1a/1b) of the coronavirus genome (Fig. 2A to fig 2C) to identify thresholds on pair-wise patristic distances (PPD) that demarcate virus clusters at different ranks.

Figure 2 A ORF of SARS-CoV Concatenated MSAs (multiple sequence alignments) of the protein domain

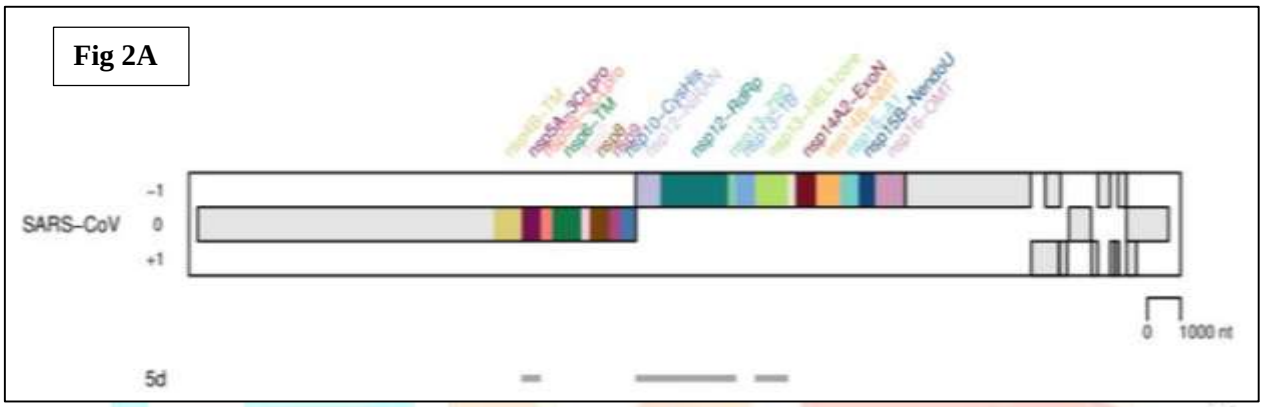
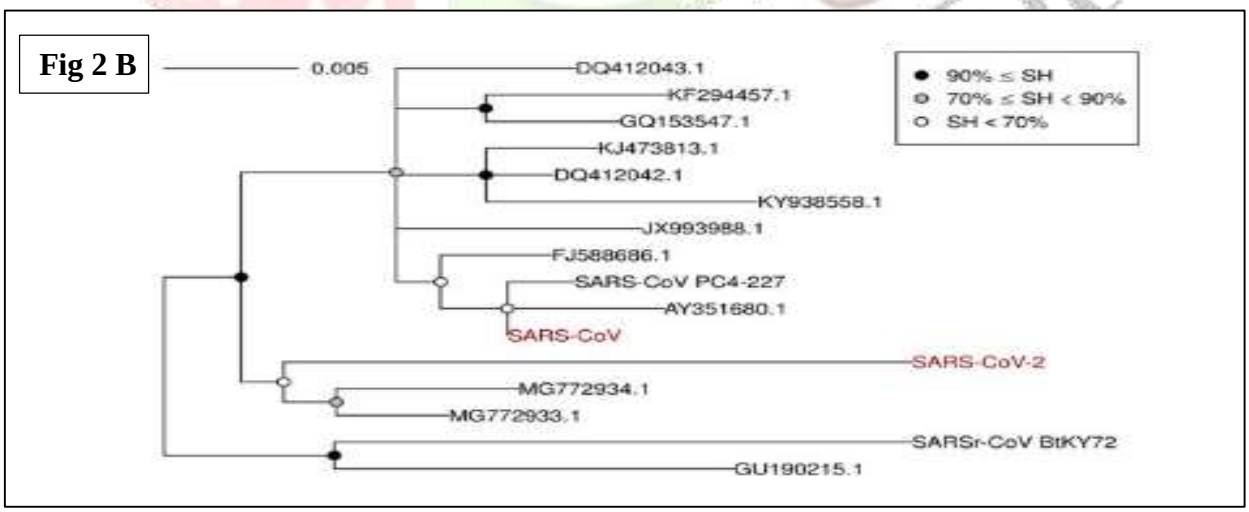


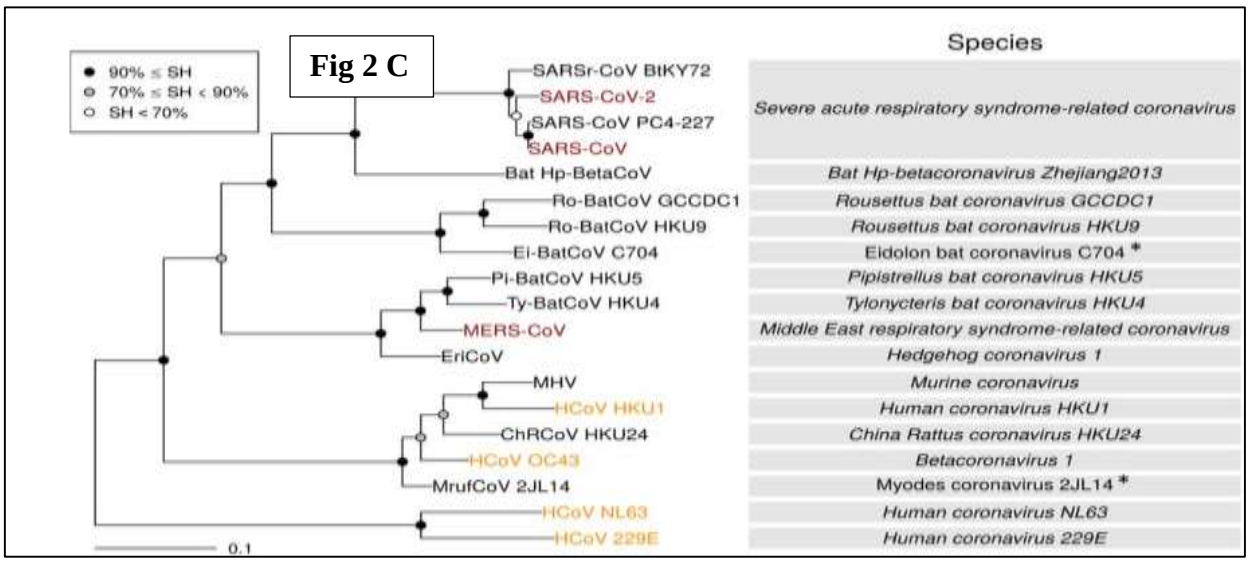
Fig (2A) Concatenated MSAs (multiple sequence alignments) of the protein domain combination used for phylogenetic and DEmARC ("*D*iv*E*rsity p*A*rtitioning by hie*R*archical *C*lustering") analyses of the Coronaviridae family. Shown are the locations of the replicative domains conserved in the Nidovirales order (5d, 5 domains: 3CLpro, 3C-like protease; NiRAN, nidovirus RdRp-associated nucleotidyltransferase; RdRp, RNA-dependent RNA polymerase; HEL1, superfamily 1 helicase with upstream Zn-binding domain (ZBD)) in relation to several other ORF1a/b-encoded domains and other major open reading frames in the SARS-CoV genome.

(2**B**) The Maximum-Likelihood (ML) tree of the species *SARS related coronavirus*



(2**B**) The Maximum-Likelihood (ML) tree of the species *Severe acute respiratory syndrome-related coronavirus* was reconstructed by IQ-Tree 1.6.1 using 83 sequences with the best fitting evolutionary model. Subsequently, the tree was purged from the most similar sequences and midpoint-rooted. Branch support was estimated using the Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT) with 1000 replicates. GenBank IDs for all viruses except four are shown; SARS-CoV, AY274119.3 35; SARS-CoV-2 MN908947.3; SARSr-CoV_BtKY72, KY352407.1; SARS-CoV_PC4-227, AY613950.1

Figure 2 C : IQ ML Tree for Coronaviruses



(2**C**) Shown is an IQ-Tree ML tree of single virus representatives of thirteen species and four representatives of the species *Severe acute respiratory syndrome-related coronavirus*, genus *Betacoronavirus*. The tree is rooted with HCoV-NL63 and HCoV-229E, representing two species of the genus *Alphacoronavirus*. Red, zoonotic viruses with varying pathogenicity in humans; orange, common respiratory viruses that circulate in humans. Asterisk, ICTV (Committee on Taxonomy of Viruses) approval for the two coronavirus species with non-italicized names is pending. The species differentiation threshold/limit in the family Coronaviridae is defined/imposed by viruses whose PPD may cross the inter-species demarcation threshold. Due to their minute share of $\sim 10^{-4}$ of the total number of all intra- and inter-species PPDs, they may not even be visually recognized in a conventional diagonal plot clustering viruses on species basis (Fig. 3A) 36.

Figure 3. Species Pairwise Distance Demarcation In The Family Coronaviridae.

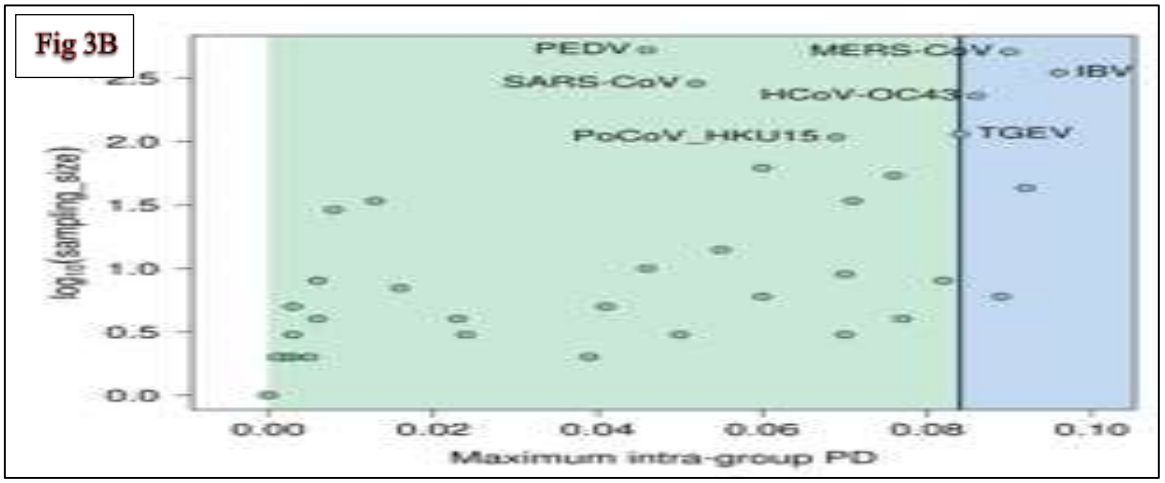
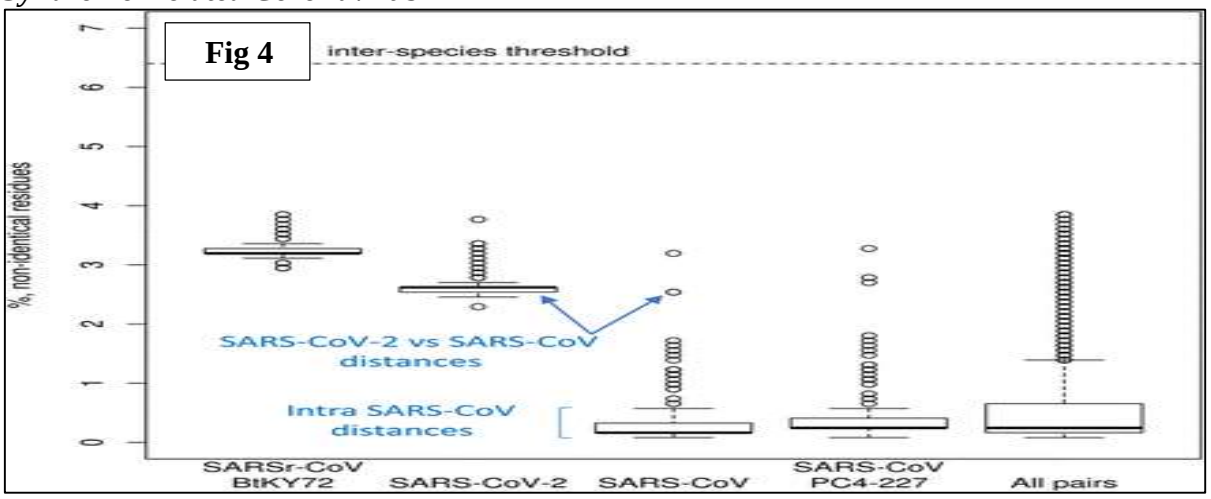


Fig 3 (**A**) Diagonal matrix of PPDs of 2505 viruses clustered according to 49 coronavirus species and ordered from the most to the least populous species, from left to right; green and white, PPDs smaller and larger than the inter-species threshold (panel B), respectively. Areas of the green squares along the diagonal are proportional to the virus sampling of the respective species, and virus prototypes of the five most sampled species are specified to the left; asterisk, selected species including viruses whose some PPDs crossed threshold ("violators"). Violators of the inter-species threshold appear as white dots on the green squares along the diagonal and green dots off the diagonal, respectively; as there are just 656 dots of this kind (out of a total of 6,275,025 dots) in the panel, they may not even be visible; this is an indication of the strong support for intra-species virus clustering.
(**B**) Maximal intra-species PPDs (X axis, linear scale) plotted against virus sampling (Y axis, log scale) for 49 species (green dots) of the *Coronaviridae*. Indicated are the acronyms of virus prototypes of the seven most sampled species. Green and blue plot sections, intra-species and intra-subgenera PPD ranges. Vertical black line represents inter-species threshold in fig 3B.

Figure 4. Pairwise Distances between Selected Viruses within the Species *Severe Acute Respiratory Syndrome-Related Coronavirus*



Intra-species PDs of SARS-CoV-2 belong to the top 25% of this species and also include the largest PD, that between SARS-CoV-2 and an African bat virus isolate (SARSr-CoV_BtKY72)**37**

(**Fig. 4**), representing two basal lineages within the species *Severe acute respiratory syndrome-related coronavirus* that constitute very few known viruses (**Fig. 2BC**). These relationships stand in contrast to the shallow branching of the most populous lineage of this species which includes all the human SARS-CoV isolates collected during the 2002-2003 outbreak and the closely related bat viruses of Asian origin identified in the search for the potential zoonotic source of that epidemic**38**. (Hu, B. et al. 2017) The current sampling defines a very small median PD for human SARS-CoVs, which is approximately 15 times smaller than the median PD determined for SARS-CoV-2 (0.16% vs 2.6%, **Fig. 4**) 36. This small median PD of human SARS-CoVs also dominates the species-wide PD distribution (0.25%, **Fig. 4**). Along with the initial failure to detect the causative agent of the disease using SARS-CoV-specific PCR setups, the separation from SARS-CoV in the phylogeny and the PD space explains why 2019-nCoV (SARS-CoV-2) may be considered a novel virus by many researchers.

Figure 5. Virus Novelty and Naming of the Three Zoonotic Coronaviruses Emerging In the First Decades of the 21st Century

Fig. 5

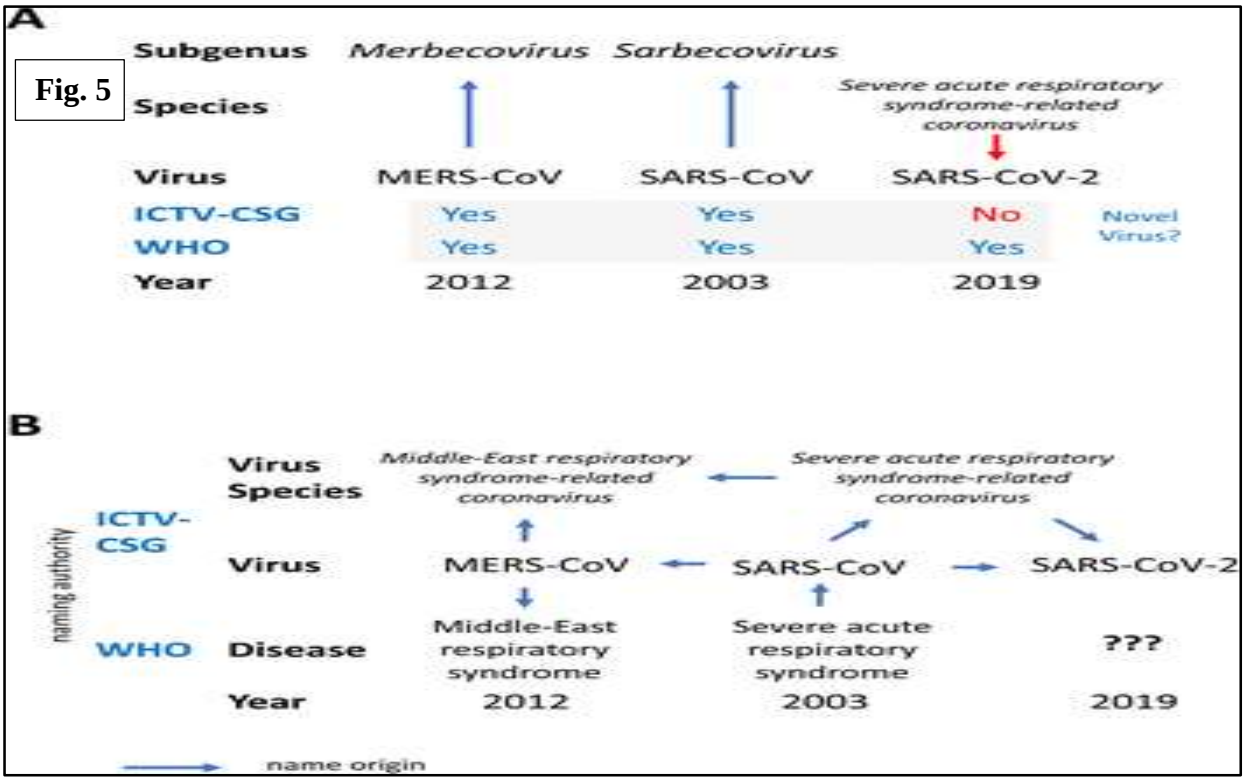
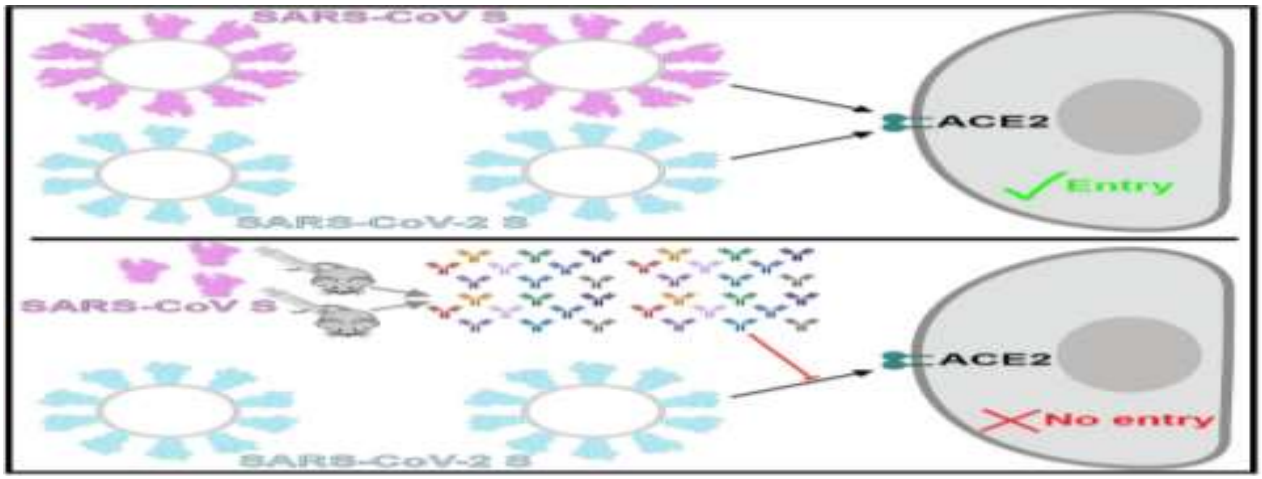


Fig 5: Year indicates the year in which the virus was first identified. (A) Independent assessments of virus novelty by the ICTV-CSG (International Committee on Taxonomy of Viruses - Coronavirus Study Group) and WHO (World Health Organisation) performed during the three outbreaks came to different conclusions. Vertical arrows indicate the degree of virus novelty according to taxonomy. (B) History of coronavirus naming during the three zoonotic outbreaks in relation to virus taxonomy and disease (clinical manifestation).

In contrast to *SARS-CoV*, the name *SARS-CoV-2* has NOT been derived from the name of the SARS disease (**Fig. 5B**) 36, and in no way, it should be used to predefine the name of the disease (or spectrum of diseases) caused by *SARS-CoV-2* in humans, which will be decided upon by the WHO. The available yet limited epidemiological and clinical data for *SARS-CoV-2* suggest that the disease spectrum and transmission modes of this virus and *SARS-CoV* may differ **18**. Also, the diagnostic methods used to confirm *SARS-CoV-2* infections are not identical to those of *SARS-CoV*. This is reflected by the specific recommendations for public health practitioners, healthcare workers and laboratory diagnostic staff for *SARS-CoV-2/2019-nCoV* **39** (e.g. WHO guidelines for *2019-nCoV*; https://www.who.int/emergencies/diseases/novel-coronavirus-2019). In this framework, the emergence of *SARS-CoV-2* as a human pathogen in December 2019 may be perceived as completely independent from the *SARS-CoV* outbreak in 2002-2003. Although *SARS-CoV-2* is NOT a descendent of *SARS-CoV* **(Fig. 2B) 36** and the introduction of each of these viruses into humans was likely facilitated by unknown external factors, the two viruses are genetically so close to each other **(Fig. 2C) 36** that their evolutionary histories and characteristics are mutually informative. To connect this development to health care, diagnostic tools that target the entire species should complement existing tools that detect individual pathogenic variants.

TWO NOTABLE GENOMIC FEATURES OF *SARS-COV-2*

Figure 6: Notable Features of the *SARS-Cov-2* Genome



On the basis of structural studies [37,38], [39] and Biochemical experiments[1,40,41] *SARS-CoV-2* seems to have an RBD (receptor-binding domain) that binds with high affinity to ACE2 from humans, ferrets, cats and other species with high receptor homology [42].

A. *SARS-CoV-2* appears to be optimized for binding to the human receptor ACE2 (Angiotensin converting enzyme 2); that means SARS-CoV-2 binds with high affinity to human ACE2 and uses it as an entry receptor to invade target cells. Zheng-Li Shi, a virologist at the Wuhan Institute of Virology in China, and her colleagues analysed samples of the new virus from seven patients who had been admitted to a hospital in late December 2019. The researchers isolated the virus from one patient and used it to infect cells grown in a laboratory. When laboratory cells had the ACE2 protein on their surface, the virus was able to break into them. The virus could use ACE2 proteins from humans to get into cells, as well as human cells with ACE2 proteins and also enzyme extracted from Chinese horseshoe bats, civets and pigs. Shi and her team also discovered that the spiky protein that *2019-nCoV* uses to attach to ACE2 have some additional pieces compared with its counterpart on the SARS virus.

B. Coronavirus transmembrane spike (S) glycoproteins forms homotrimers protruding from the viral surface (surface exposed) which mediate virus entry into host cells. The spike protein of *SARS-CoV-2* has a functional polybasic (furin and other proteases-PRAR) cleavage site at the S_1/S_2 boundary which is cleaved during biosynthesis—a novel feature setting this virus apart from *SARS-CoV* and *SARSr-CoVs*. (Fig.7b) A leading proline is inserted at this site in *SARS-CoV-2* S glycoproteins; thus, the inserted sequence is PRRA (Fig. 7b) [43] The turn created by the proline is predicted to result in the addition of O-linked glycans to S673, T678 and S686, which flank the cleavage site and are unique to *SARS-CoV-2* (Fig. 7b). S1 subunit is responsible for binding to the host cell receptor and S2 subunit is responsible for fusion of the viral and cellular membranes. The distal S_1 subunit comprises the receptor-binding domain (RBD) and contributes to stabilization of the pre fusion state of the membrane-anchored S_2 subunit that contains the fusion machinery [44] [45] [46] [47] [48] [49] [50]. The RBD in the spike protein is the most variable part of the coronavirus genome [1] [2]. Six RBD amino acids have been shown to be critical for binding to ACE2 receptors and for determining the host range of SARS-CoV-like viruses [42]. With coordinates based on *SARS-CoV*, they are Y442, L472, N479, D480, T487 and Y4911, which correspond to L455, F486, Q493, S494, N501 and Y505 in *SARS-CoV-2* [42]. Five of these six residues differ between *SARS-*

CoV-2 and *SARS-CoV* (Fig. 7a).This polybasic furin cleavage has been proposed to activate the protein for membrane fusion via extensive irreversible conformational changes 51 52 53 54 55 56. As a result, coronavirus entry into susceptible cells is a complex process that requires the concerted action of receptor-binding and proteolytic processing of the S protein to promote virus-cell fusion. Cumulative function of S1 and S2 subunits during biogenesis of *SARS-CoV-2* sets this virus apart from *SARS-CoV* and *SARS-related CoVs*. That means Coronavirus entry into host cells is mediated by the transmembrane spike (S) glycoprotein that forms homotrimers protruding from the viral surface 57. Different coronaviruses use distinct domains within the S_1 subunit to recognize a variety of attachment and entry receptors, depending on the viral species. Endemic human coronaviruses *OC43* and *HKU1* attach via their S domain A (S^A) to 5-N-acetyl-9-*O*-acetyl-sialosides found on glycoproteins and glycolipids at the host cell surface to enable entry into susceptible cells 58 59 60. *MERS-CoV* S, however, uses domain A to recognize non-acetylated sialoside attachment receptors 61 62, which likely promote subsequent binding of domain B (S^B) to the entry receptor, dipeptidyl-peptidase 63 64 . *SARS-CoV* and several SARS-related coronaviruses (*SARSr-CoV*) interact directly with ACE2 via S^B to enter target cells 65 45 66 67 47 68. The *SARS-CoV-2* S glycoprotein shares 76% amino acid sequence identity with the *SARS-CoV* S Urbani and 80% identity with bat *SARSr-CoV* ZXC21 S and ZC45 S glycoproteins. The latter two *SARSr- CoV* sequences were identified from *Rinolophus sinicus* (Chinese horseshoe bats), the species from which *SARSr-CoV* WIV-1 and WIV-16 were isolated 65 68. Furthermore, Zhou et al.1 (2020) recently reported that *SARS-CoV-2* is most closely related to the bat *SARSr-CoV RaTG13*, with which it forms a distinct lineage from other SARSr-CoVs, and that their S glycoproteins share 97% amino acid sequence identity.

To investigate the functional determinants of S-mediated entry into target cells pseudotyping system using a *Murine Leukaemia Virus* (MLV) was set out by J.K. Millet, G.R. Whittaker in 2016. For *in vitro* assessment of the ability of SARS-CoV-2 S to promote entry into target host cells, transduction of SARS-CoV-2 S-MLV and SARS-CoV S-MLV into VeroE6 (African green monkey) cells was carried out by Alexandra C. Walls in 2020. Vero cells are interferon-deficient; unlike normal mammalian cells, they do not secrete interferon alpha or beta when infected by viruses.69 However, they still have the interferon-alpha/beta receptor, so they respond normally when recombinant interferon is added to their culture media. Conclusion was that both pseudoviruses entered cells equally well (Figure 7A) 70, suggesting that *SARS-CoV-2 S-MLV* could use African green monkey ACE2 as entry receptor. These results were confirmed by using BHK (Baby hamster kidney) cells and observed that transient transfection with hACE2 ((human angiotensin-converting enzyme 2) rendered them susceptible to transduction with *SARS-CoV-2 S-MLV* 70 (Figure 8B to fig 8D). Finally these results demonstrate *SARS-CoV-2* S mediates entry in VeroE6 cells and in BHK cells transiently transfected with human ACE2, establishing ACE2 as a functional receptor for this novel coronavirus. Cryoelectron microscopy (cryo-EM) structures of the *SARS-CoV-2* S ectodomain trimer (spike in the prefusion conformation) reveal that *2019-nCoV* adopts multiple S^B conformations that are reminiscent of previous reports on both *SARS-CoV* S and *MERS-CoV* S. (Fig.10) Pairwise protein sequence analysis of seven conserved non-structural proteins domains show that *2019-nCoV* virus belongs to the species of *SARSr-CoV* and 96% identical at the whole-genome level to a bat coronavirus. (Fig 11 to Fig 13)

Figure.7 Features of the Spike Protein in Human *SARS-Cov-2* and Related Coronaviruses

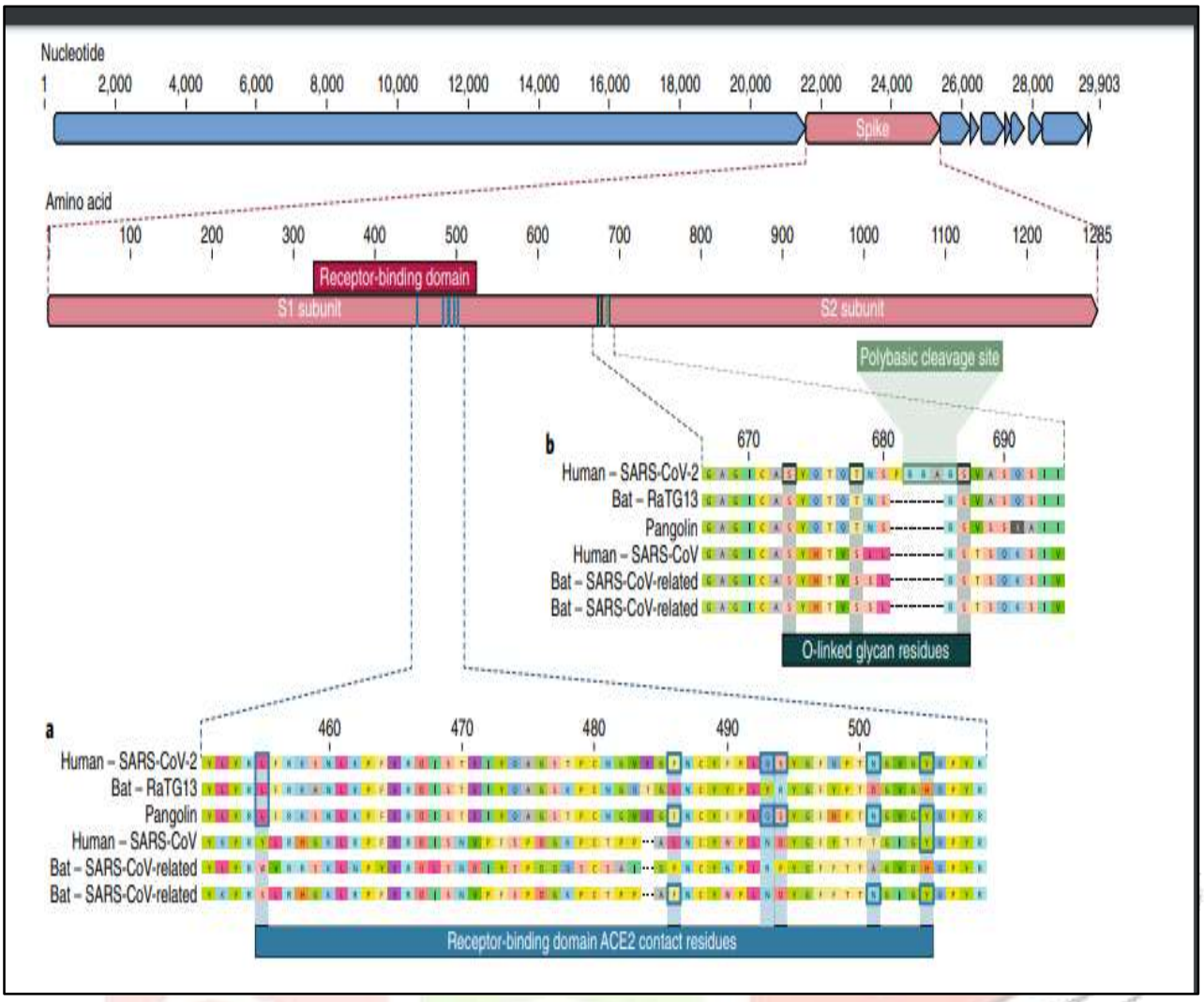


Fig 7 a, Mutations in contact residues of the *SARS-CoV-2* spike protein. The spike protein of *SARS-CoV-2* (red bar at top) was aligned against the most closely related *SARS-CoV-like coronaviruses* and *SARS-CoV* itself. Key residues in the spike protein that make contact to the ACE2 receptor are marked with blue boxes in both *SARS-CoV-2* and related viruses, including *SARS-CoV* (Urbani strain).

b, Acquisition of polybasic cleavage site and O-linked glycans. Both the polybasic cleavage site and the three adjacent predicted O-linked glycans are unique to *SARS-CoV-2* and were not previously seen in lineage B *Betacoronaviruses*. Sequences shown are from NCBI GenBank, accession codes MN908947, MN996532, AY278741, KY417146 and MK211376. The *Pangolin coronavirus* sequences are a consensus generated from SRR10168377 and SRR10168378 (NCBI BioProject PRJNA573298) 71 72.

Figure 8. ACE2 Is a Functional Receptor for *SARS-CoV-2* S

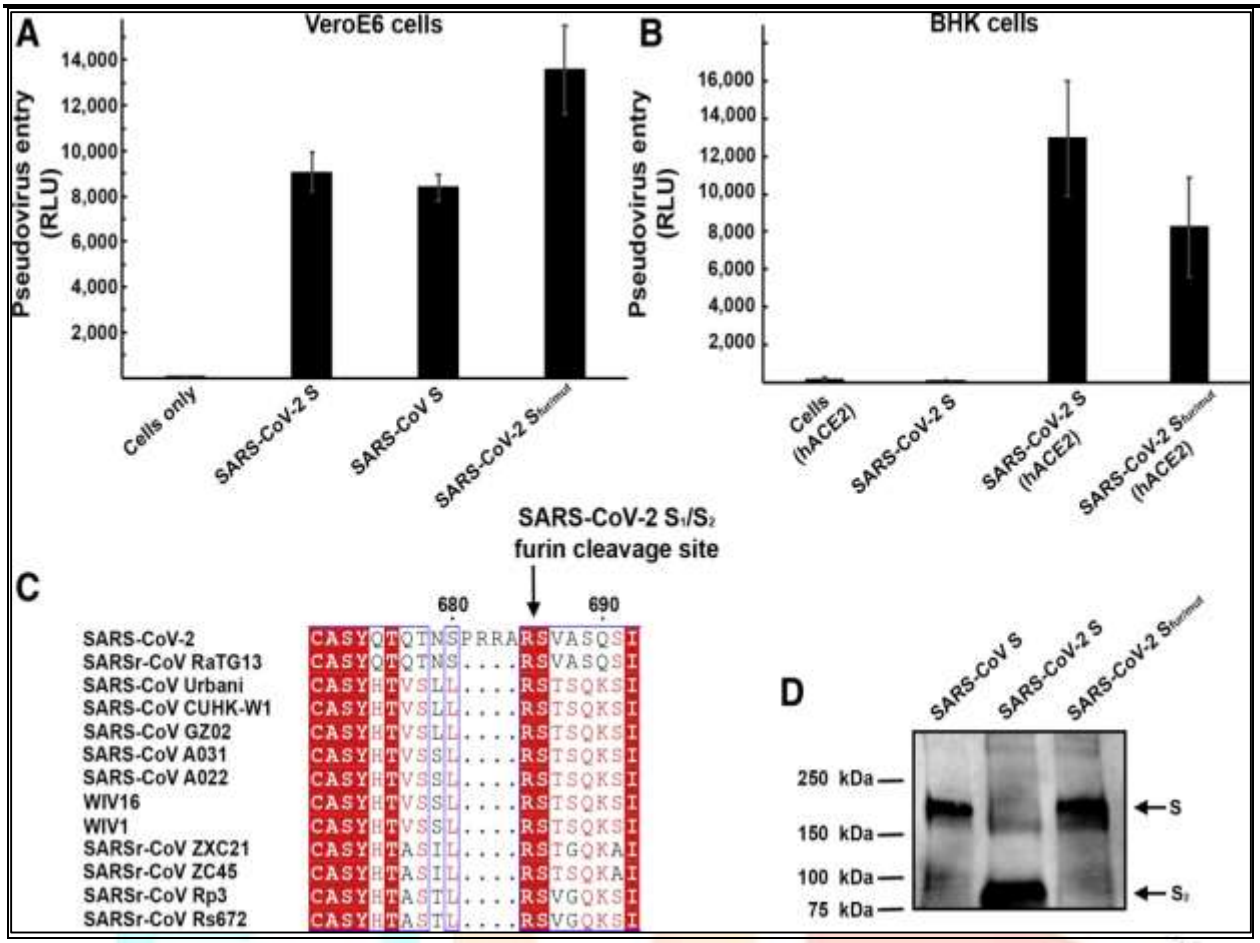


Fig 8. (A) Entry of *MLV* pseudotyped with *SARS-CoV-2* S, *SARS-CoV* S and *SARS-CoV-2* S $_{fur/mut}$ in VeroE6 cells. Data are represented as mean ± standard deviation of technical triplicates.

(B) Entry of *MLV* pseudotyped with *SARS-CoV-2* S or *SARS-CoV-2* S$_{fur/mut}$ in BHK cells transiently transfected with hACE2. The experiments were carried out with two independent pseudovirus preparations and a representative experiment is shown. Data are represented as mean ± standard deviation of technical triplicates.

(C) Sequence alignment of *SARS-CoV-2* S with multiple related *SARS-CoV* and *SARSr-CoV* S glycoproteins reveals the introduction of an S_1/S_2 furin cleavage site in this novel coronavirus. Identical and similar positions are respectively shown with white or red font. The four amino acid residue insertion at *SARS-CoV-2* S positions 681-684 is indicated with periods.

(D) Western blot analysis of *SARS-CoV S-MLV*, *SARS-CoV-2 S-MLV*, and *SARS-CoV-2* $S_{fur/mut}$*-MLV pseudovirions*.

Figure 9. *SARS-CoV-2* S Recognizes hACE2 with Comparable Affinity to *SARS-CoV* S

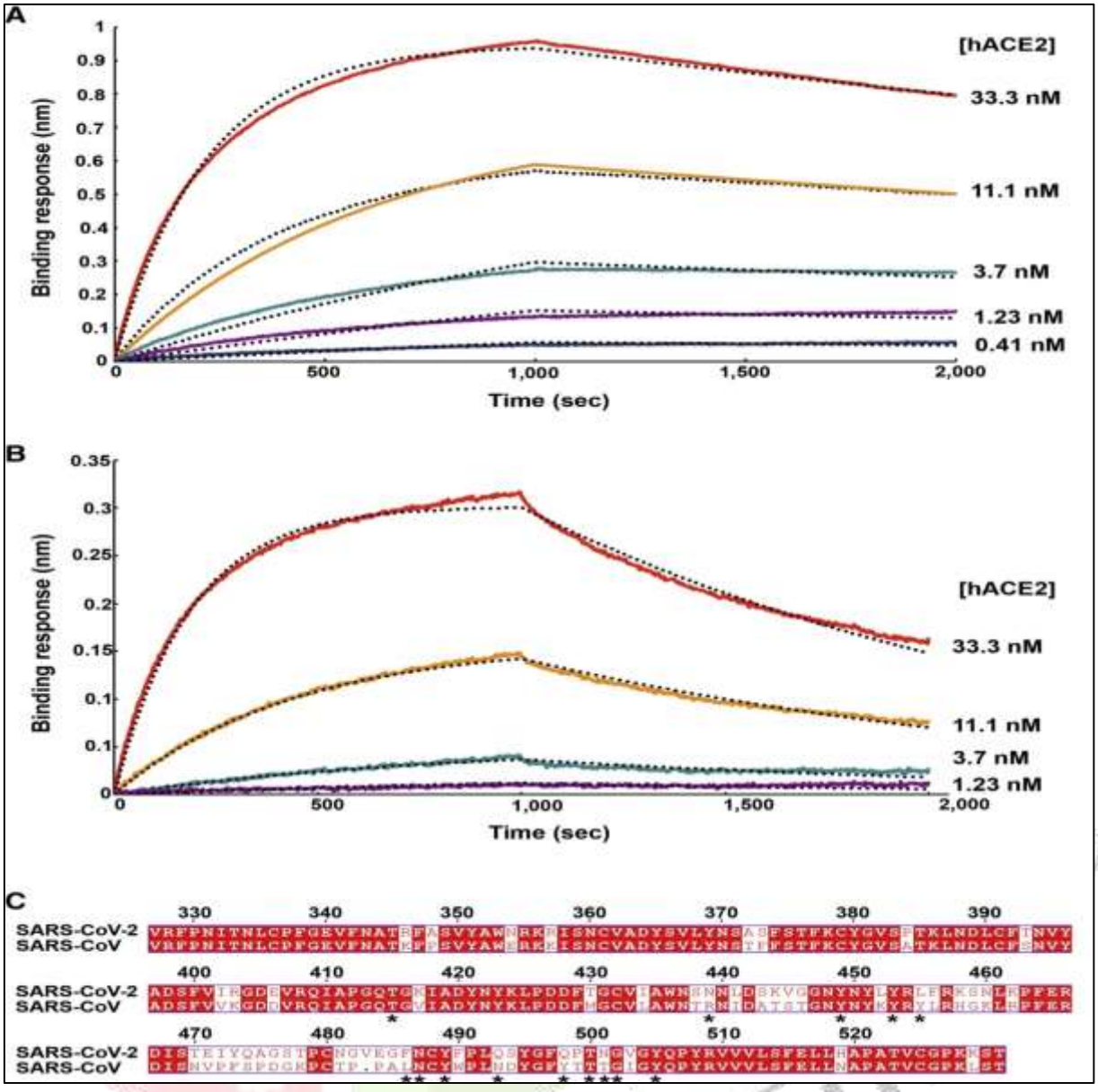


Fig 9. (A and B) Biolayer interferometry binding analysis of the hACE2 ectodomain to immobilized *SARS-CoV-2* S^B (A) or *SARS-CoV* S^B (B). The experiments were repeated with different protein preparations and one representative set of curves is shown. Dotted lines correspond to a global fit of the data using a 1:1 binding model.

(C) Sequence alignment of *SARS-CoV-2* S^B and *SARS-CoV* S^B *Urbani* (late phase of the 2002–2003 SARS-CoV epidemic). Identical and similar positions are respectively shown with white or red font. The single amino acid insertion at position 483 of the *SARS-CoV-2* S^B is indicated with a period at the corresponding *SARS-CoV* S^B position. The 14 residues that are key for binding of *SARS-CoV* S^B to hACE2 are labelled with a star.

Figure. 10 Cryo-EM Structure of the *2019-Ncov* Spike in the Prefusion Conformation

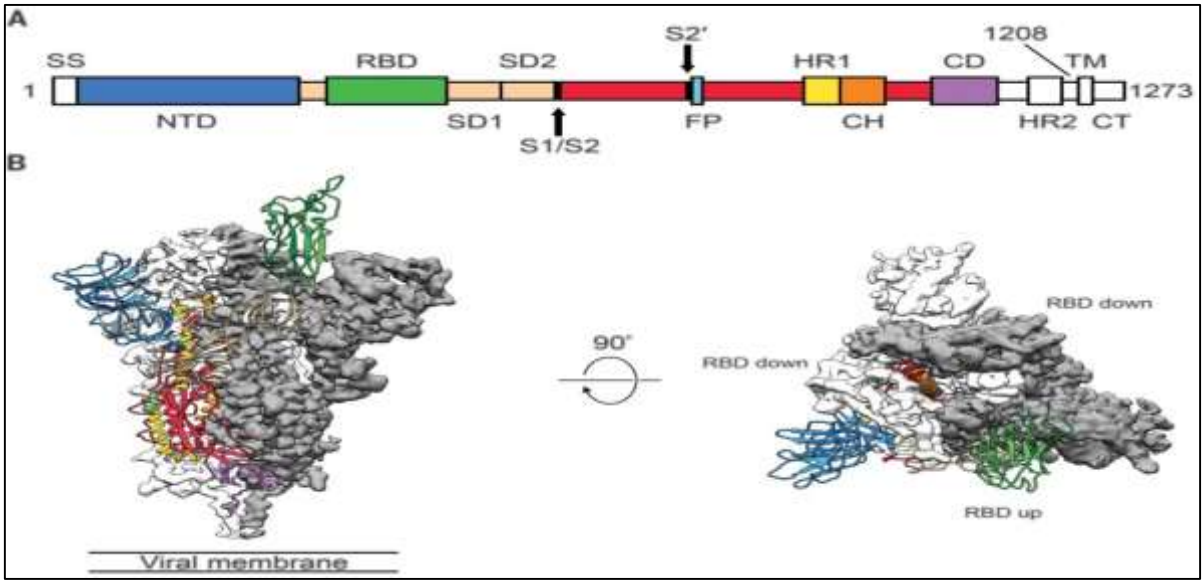
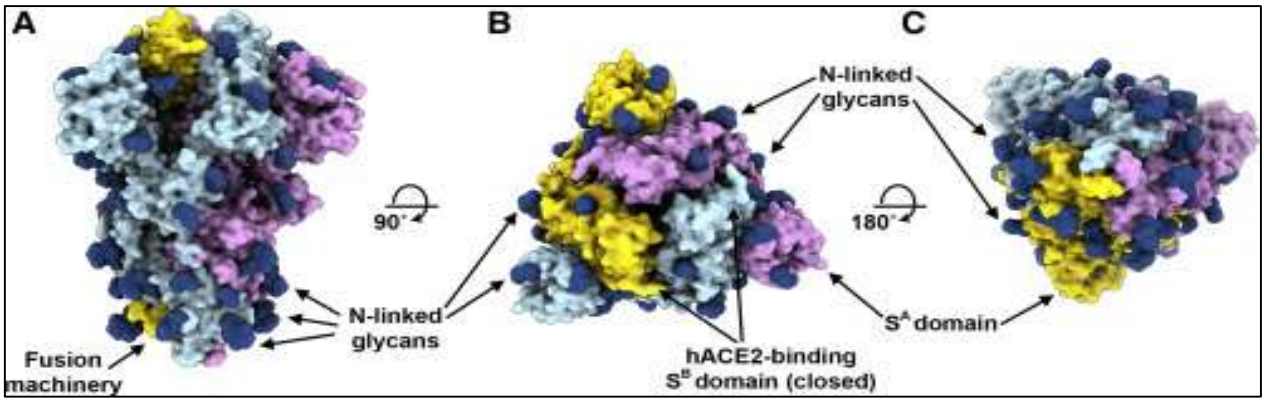


Fig 10 (**A**) Schematic of *2019-nCoV* S primary structure colored by domains. Domains that were excluded from the ectodomain expression construct or could not be visualized in the final map are colored white. SS, signal sequence; S2′, S2′ protease cleavage site; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane domain; CT, cytoplasmic tail. Arrows denote protease cleavage sites.

(**B**) Side and top views of the prefusion structure of the *2019-nCoV* S protein with a single RBD in the up conformation. The two RBD down protomers are shown as cryo-EM density in either white or gray and the RBD up protomer is shown in ribbons colored corresponding to the schematic in (A).

Figure 11. Comparisons of the *SARS-CoV-2* and *SARS-CoV* S Structures

Figure 12. Organization of the *SARS-CoV-2* S N-Linked Glycans



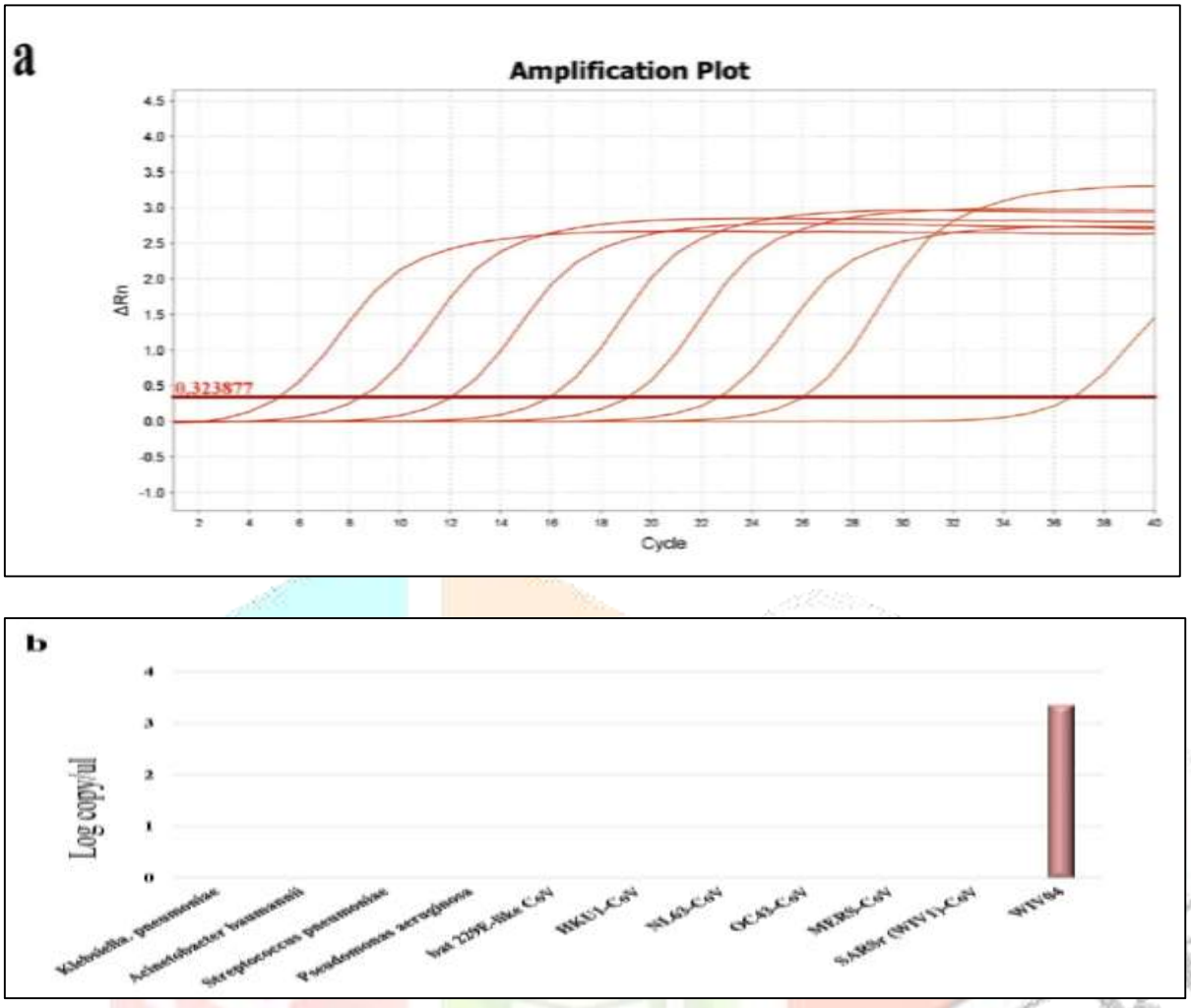
12 (A–C) Ribbon diagrams of the *SARS-CoV-2* S closed structure rendered as a surface with glycans resolved in the cryo-EM map rendered as dark blue spheres.

MOLECULAR METHODS USED TO DETECT *2019-nCOV*

New coronavirus (*2019-nCoV*), which is a major cause of an epidemic of acute respiratory syndrome in humans originated in Wuhan, China, started on 12 December 2019. Typical clinical symptoms of these patients were fever, dry cough, breathing difficulties (dyspnoea), headache and pneumonia. In more severe cases disease inception which can cause progressively respiratory failure due to alveolar damage (as observed by transverse chest computerized-tomography images) and ultimately death. Samples from seven patients with severe pneumonia (six of whom are sellers or deliverymen from the seafood market), who were admitted to the intensive care unit of Wuhan Jin Yin-Tan Hospital at the beginning of the outbreak, were sent to the laboratory at the Wuhan Institute of Virology (WIV) for the diagnosis of the causative pathogen . As a laboratory investigating CoV, first used pan-CoV PCR primers to test these samples 73, given that the outbreak occurred in winter and in a market—the same environment as SARS infections. Five samples were PCR-positive for CoVs. One sample (WIV04), collected from the bronchoalveolar lavage fluid (BALF), was analysed by metagenomics analysis using next-generation sequencing (NGS) to identify potential aetiological agents. Of the 10,038,758 total reads—of which 1,582 total reads were retained after filtering of reads from the human genome—1,378 sequences matched the sequence of *SARSr-CoV* (Fig.15a) (i.e. 87.1% sequence identity to SARS-CoV). It was observed that by *de novo* assembly and targeted PCR, 29,891-base-pair CoV genome shared 79.6% sequence identity to *SARS-CoV BJ01* (GenBank accession number AY278488.2). High genome coverage was obtained by remapping the total reads to this genome. This full length genome sequence has been submitted to GISAID (https://www.gisaid.org/) (accession number EPI_ISL_402124). Following the name given by World Health Organization (WHO), virus was named as *novel coronavirus 2019* (*2019-nCoV*). Four additional full-length genome sequences of *2019-nCoV* (WIV02, WIV05, WIV06 and WIV07) (GISAID accession numbers EPI_ISL_402127–402130) were more than 99.9% identical to each other using next-generation sequencing and PCR. The virus genome consists of six major open-reading frames (ORFs) that are common to coronaviruses and a number of other accessory genes (Fig. 15) 1. Further analysis indicates that some of the *2019-nCoV* genes shared less than 80% nucleotide sequence identity to *SARS-CoV*. However, the amino acid sequences of the seven conserved replicase domains in ORF1ab that were used for CoV species classification were 94.4% identical between *2019-nCoV* and *SARS-CoV*, suggesting that the two viruses belong to the same species, *SARSr-CoV*. For serological detection of *2019-nCoV*, by ELISA (enzyme-linked immunosorbent assays) method previously developed nucleocapsid (N) protein from bat *SARSr-CoV Rp3* was used as an antigen for IgG and IgM . This protein shared 92% amino acid identity to N protein of *2019-nCoV* and showed no cross-reactivity against other human coronaviruses except *SARSr-CoV* 74. From five serum samples with viral infections antibody levels in one patient (ICU-06) was monitor for 7, 8, 9 and 18 days after the onset of disease. A clear trend was observed in the IgG and IgM titres, which increased over time, except that the IgM titre was decreased in the last sample (Fig. 16b) 1. In second round, serum of the 7 virus-positive patients around 20 days after disease onset for the presence of viral antibodies was tested by ELISA and it was observed that all patient

samples were strongly positive for viral IgG 84 (Fig. 16b) 1. There were also three IgM-positive samples, indicating an acute infection.

Figure. 13: Molecular Detection Method Used To Detect *2019-Ncov*.



a, Standard curve for qPCR primers using the HiScript II One Step qRT–PCR SYBR Green Kit (Vazyme Biotech) The PCR product of the *S* gene that was serial diluted in the range of 10^8 to 10^1 (lines from left to right) was used as a template.

b, Specificity of the qPCR primers. Nucleotide samples from the indicated pathogens were used. 1 (From: A pneumonia outbreak associated with a new coronavirus of probable bat origin)

Figure 14: Genome Characterization Of *2019-Ncov*.

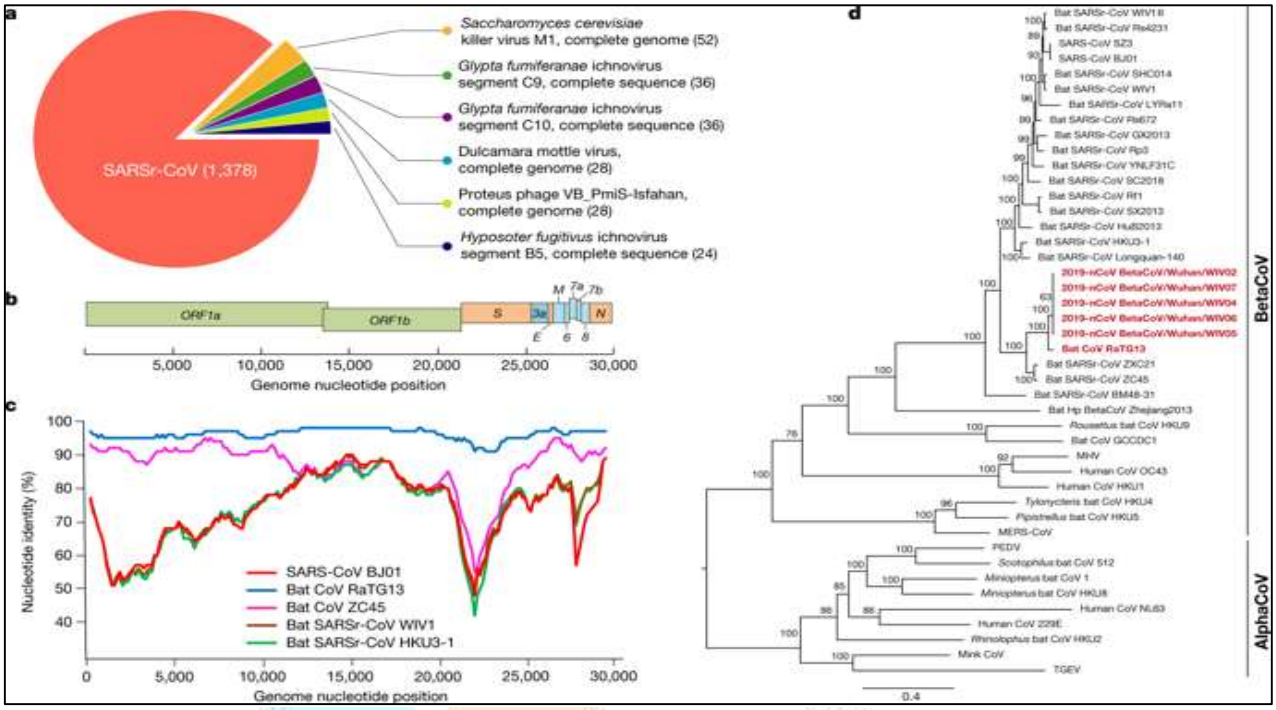


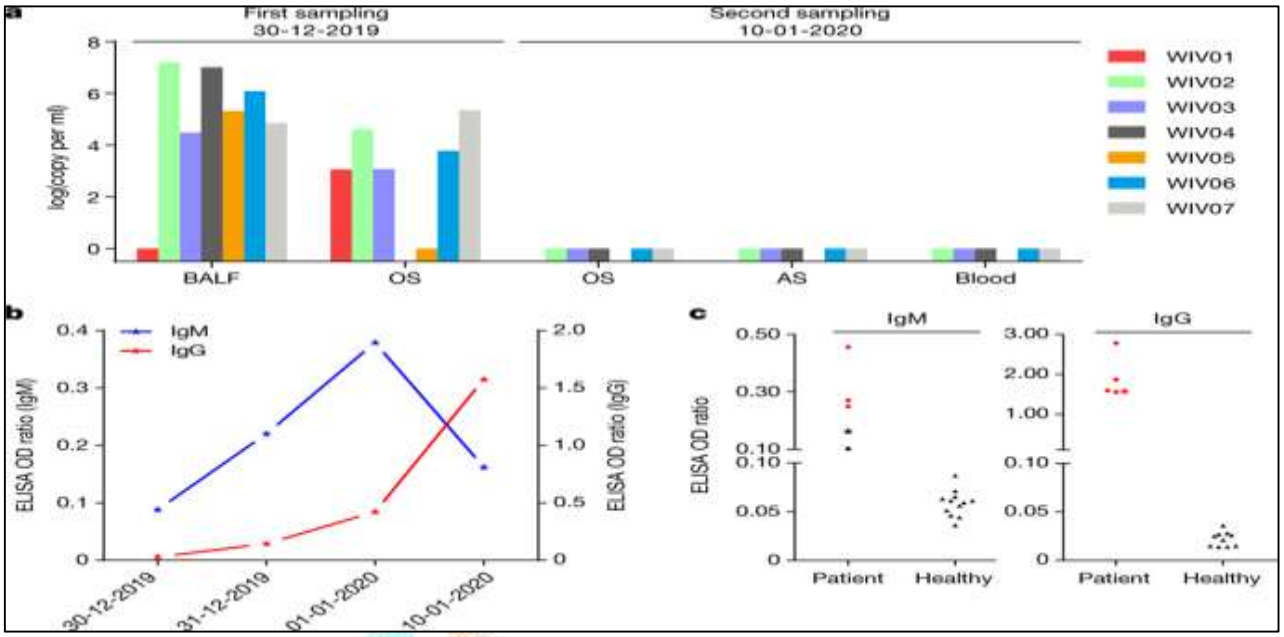
Fig. 14 a, Metagenomics analysis of next-generation sequencing of BALF from patient ICU06.

b, Genomic organization of *2019-nCoV WIV04*

c, Similarity plot based on the full-length genome sequence of *2019-nCoV WIV04*. Full-length genome sequences of *SARS-CoV BJ01*, *bat SARSr-CoV WIV1*, *bat coronavirus RaTG13* and *ZC45* were used as reference sequences.

d, Phylogenetic tree based on nucleotide sequences of complete genomes of coronaviruses- *MHV, murine hepatitis virus; PEDV, porcine epidemic diarrhoea virus; PTGEV, porcine transmissible gastroenteritis virus*. The scale bars represent 0.1 substitutions per nucleotide position. (NGS- using BGI MGISEQ2000 and Illumina MiSeq 3000 sequencers based on the bioinformatics platform MGmapper (PE_2.24 and SE_2.24), Microsoft Office 2010, Geneious v.11.0.3 and MEGAHIT v.1.2.9 SMARTer RACE 5′/3′ kit (Takara). Genomes were annotated using the Clone Manager Professional Suite 8 (Sci-Ed Software).

Fig. 15: *In Vitro* Cell Infection and Serological Investigation of Patient Samples



a, Molecular detection of *2019-nCoV* in seven patients. AS, anal swab; OS, oral swab. Vero E6 and Huh7 Cell lines cultured in DMEM + 10% FBS+ 16 μg ml^{-1} trypsin added with penicillin (100 units ml^{-1}) and streptomycin (15 μg ml^{-1}) used for virus isolation.

b, Dynamics of *2019-nCoV* antibody levels in one patient who showed signs of disease on 23 December 2019 (ICU-06). OD ratio, optical density at 450–630 nm. The right and left Y axes indicate ELISA OD ratios for IgM and IgG, respectively.

c, Serological test of *2019-nCoV* antibodies in five patients . The asterisk indicates data collected from patient ICU-06 on 10 January 2020.

b, **c**, The cut-off was to 0.2 for the IgM analysis and to 0.3 for the IgG analysis, according to the levels of healthy controls.

CONCLUSIONS

Severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*), caused an illness known as COVID-19, which was similar to SARS and was being characterized primarily by fever and respiratory symptoms. The virus was likewise highly contagious. By early 2020 it had spread throughout regions of China and had reached the United States, Europe and all over the world having been carried by travellers from affected provinces. In March 2020 the World Health Organization declared the outbreak a pandemic.

In conclusion, Coronaviruses incline to be species specific, yet SARS-CoV seems to have crossed species barriers and diseased humans, resulting in great morbidity and mortality. The virus emerged in humans in 2002; it likely jumped to humans from an animal reservoir, believed to be horseshoe bats. The ability of *SARS coronavirus* to jump to humans certainly required genetic changes in the virus. These changes are supposed to have transpired in the palm civet, since the *SARS virus* present in horseshoe bats is unable to infect humans directly. The novel *MERS coronavirus* was known to have originated in bats and was thought to be passed from bats to other animals before being transmitted to humans. Although very first cases of the CoVID-19 disease were linked to direct exposure to the Huanan Seafood Wholesale Market of Wuhan, the animal-to-human

transmission was assumed as the main mechanism. But now it is concluded that the virus could also be transmitted from human-to-human, and symptomatic people are the most frequent source of COVID-19 spread.

At present, no specific antiviral managements or vaccines are available to battle any human coronavirus. Studying coronaviruses will therefore help in understanding the principles governing cross-species transmission and adaptation to humans and in preparing prevention of future zoonotic outbreaks.

Acknowledgements

Authors thank State and local health departments; clinical staff members caring for patients, India. Thank all those who have contributed sequences to the GISAID database (https://www.gisaid.org/) and analyses to Virological.org (http://virological.org/)

Conflicts of Interest: The authors declare no conflict of interest.

.

REFERENCES

1. Zhou, P., Yang, X., Wang, X. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579**,** 270–273 (2020). https://doi.org/10.1038/s41586-020-2012-7

2. Wu, F., Zhao, S., Yu, B. *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* 579**,** 265–269 (2020). https://doi.org/10.1038/s41586-020-2008-3

3. Gorbalenya, A. E. et al. The new scope of virus taxonomy: partitioning the virosphere into 15 hierarchical ranks. *Nat Microbiol in press (*2020*).*

4.

5. Jiang S, Shi Z, Shu Y, et al. A distinct name is needed for the new coronavirus. Lancet 2020; published online Feb 19. https://doi.org/ 10.1016/S0140-6736(20)30419-0.

6. Tyrrell, D. A. J., and Bynoe, M. L., 1965, Cultivation of a novel type of common cold virus in organ culture, *Br. Med. J.* 1**:**1467.

7. Tyrrell, D. A. J., Almeida, J. D., Berry, D. M., Cunningham, C. H., Hamre, D., Hofstad, M. S., Malluci, L., and Mcintosh, K., 1968a, Coronaviruses, *Nature* 220**:**650.

8. Tyrrell, D. A. J., Bynoe, M. L., and Hoorn, B., 1968b, Cultivation of "difficult" viruses from patients with common colds, *Br. Med. J.* **1:**606.

9. Tyrrell, D. A. J., Mika-Johnson, M., Philips, G., Douglas, W. H. J., Chappie, P. J., 1979, Infection of cultured human type II pneumocytes with certain respiratory viruses, *Infect. Immun.* 26**:**621.

10. Tyrrell, D. A. J., Cohen, S., and Schlarb, J. E., 1993, Signs and symptoms in common colds, *Epidemiol. Infect.* 111**:**143.

11. Almeida, J.D., Waterson, A.P. & Plowright, W. The morphological characteristics of african swine fever virus and its resemblance to tipula iridescent virus. *Archiv f Virusforschung* 20**,** 392–396 (1967). https://doi.org/10.1007/BF01241958

12. Hamre, D., & Procknow, J. J. (1966). A New Virus Isolated from the Human Respiratory Tract. *Proceedings of the Society for Experimental Biology and Medicine*, *121*(1), 190–193. https://doi.org/10.3181/00379727-121-30734

13. Siddell, S. G. et al. Additional changes to taxonomy ratified in a special vote by the International Committee on Taxonomy of Viruses (October 2018). *Archives of Virology* 164, 943–946, doi: 10.1007/s00705-018-04136-2 *(*2019*).*

14. Ziebuhr, J. et al. *Proposal 2017.013S.A.v1*. Reorganization of the family Coronaviridae into two families, Coronaviridae (including the current subfamily Coronavirinae and the new subfamily Letovirinae) and the new family Tobaniviridae (accommodating the current subfamily Torovirinae and three other subfamilies), revision of the genus rank structure and introduction of a new subgenus *rank (*2017*).*<https://talk.ictvonline.org/ICTV/proposals/2017.013S.A.v1.Nidovirales.zip.>.

15. Ziebuhr, J. et al. Proposal 2019.021S.Ac.v1. Create ten new species and a new genus in the subfamily Orthocoronavirinae of the family Coronaviridae and five new species and a new genus in the subfamily Serpentovirinae of the family Tobaniviridae. *(*2019*).* <*URL:* https://talk.ictvonline.org/ICTV/proposals/2019.021S.A.v1.Nidovirales.zip>.

16. A.M.Q. King, M.J. Adams, E.B. Carstens, & E.J. Lefkowitzde Groot, R. J. et al. in Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses (eds A.M.Q. King, M.J. Adams, E.B. Carstens, & E.J. Lefkowitz) 806–828 (Elsevier Academic Press, 2012).Google Scholar

17. Corman, V. M., Muth, D., Niemeyer, D. & Drosten, C. *Adv. Virus Res.* 100, 163–188 (2018).

18. Chen, N. et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*, doi: 10.1016/S0140-6736(20)30211-7 *(*2020*).*CrossRefGoogle Scholar

19. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, doi: 10.1016/S0140-6736(20)30183-5 *(*2020*).*CrossRefGoogle Scholar

20. Zhang, W. et al. Molecular basis of the receptor binding specificity switch of the hemagglutinins from both the 1918 and 2009 pandemic influenza A viruses by a D225G substitution. *J Virol* 87, 5949–5958, doi: 10.1128/JVI.00545-13 *(*2013*).*

21. Masters, P. S. The molecular biology of coronaviruses. *Advances in Virus Research* 66, 193–292 *(*2006*).*CrossRefPubMedWeb of ScienceGoogle Scholar

22. Perlman, S. & Netland, J. Coronaviruses post-SARS: update on replication and pathogenesis. *Nature Reviews Microbiology* **7**, 439–450, *doi*:DOI 10.1038/nrmicro2147 *(*2009*).*

23. Drosten, C. et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *New England Journal of Medicine* 348, 1967–1976 *(*2003*).*

24. Ksiazek, T. G. et al. A novel coronavirus associated with severe acute respiratory syndrome. *New England Journal of Medicine* 348, 1953–1966 *(*2003*).*

25. Peiris, J. S. M. et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 361, 1319–1325 *(*2003*).*

26. Zumla, A., Hui, D. S. & Perlman, S. Middle East respiratory syndrome. *Lancet* 386, 995–1007, doi: 10.1016/S0140-6736(15)60454-8 *(*2015*).*

27. Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D. M. E. & Fouchier, R. A. M. Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *New England Journal of Medicine* 367, 1814–1820 *(*2012*).*

28. Lauber, C. & Gorbalenya, A. E. Partitioning the genetic diversity of a virus family: approach and evaluation through a case study of picornaviruses. *Journal of Virology* 86, 3890–3904, doi: 10.1128/JVI.07173-11 *(*2012*).*

29. Lauber, C. et al. Mesoniviridae: a new family in the order *Nidovirales* formed by a single species of mosquito-borne viruses. *Arch.Virol.* 157, 1623–1628 *(*2012*)*

30. Rota, P. A. et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* 300, 1394–1399 *(*2003*).*

31. Gorbalenya, A. E., Lauber, C. & Siddell, S. *in Reference Module in Biomedical Sciences (*Elsevier, 2019*).* doi: 10.1016/B978-0-12-801238-3.99237-7

32. de Groot, R. J. et al. Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group. *Journal of Virology* 87, 7790–7792, doi: 10.1128/Jvi.01244-13 *(*2013*).*

33. Gorbalenya, A. E., Snijder, E. J. & Spaan, W. J. Severe Acute Respiratory Syndrome Coronavirus Phylogeny: toward Consensus. *Journal of Virology* 78, 7863–7866 *(*2004*).*

34. González, J. M., Gomez-Puertas, P., Cavanagh, D., Gorbalenya, A. E. & Enjuanes, L. A comparative sequence analysis to revise the current taxonomy of the family Coronaviridae. *Archives of Virology* 148, 2207–2235 *(*2003*).*

35. Lehmann, K. C. et al. Discovery of an essential nucleotidylating activity associated with a newly delineated conserved domain in the RNA polymerase-containing protein of all nidoviruses. Nucleic Acids Research 43, 8416–8434, doi: 10.1093/nar/gkv838 (2015).

36. He,R., Dobie,F., Ballantine,M., Leeson,A., Li,Y., Bastien,N., Cutts,T., Andonov,A., Cao,J., Booth,T.F., Plummer,F.A., Tyler,S., Baker,L. and Li,X. Analysis of multimerization of the SARS coronavirus nucleocapsid protein CONSRTM BCCA Genome Sciences Centre, British Columbia Centre for Disease Control and National Microbiology Laboratory Canada TITLE JOURNAL Biochem. Biophys. Res. Commun. 316 (2), 476-483 (2004)

37. Alexander E. Gorbalenya, Susan C. Baker, Ralph S. Baric, Raoul J. de Groot, Christian Drosten, Anastasia A. Gulyaeva, Bart L. Haagmans, Chris Lauber, Andrey M Leontovich, Benjamin W. Neuman, Dmitry Penzar, Stanley Perlman, Leo L.M. Poon, Dmitry Samborskiy, Igor A. Sidorov, Isabel Sola, John Ziebuhr *Severe acute respiratory syndrome-related coronavirus*: The species and its viruses – a statement of the Coronavirus Study Group (2020) bioRxiv 2020.02.07.937862; doi: https://doi.org/10.1101/2020.02.07.937862

38. Tao, Y. & Tong, S. X. Complete Genome Sequence of a Severe Acute Respiratory Syndrome-Related Coronavirus from Kenyan Bats. Microbiol Resour Ann 8, doi: 10.1128/MRA.00548-19 (2019).

39. Hu, B. et al. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. Plos Pathogens 13, e1006698, doi: 10.1371/journal.ppat.1006698 (2017).

40. World Health Organization (WHO) Coronavirus disease 2019 Coronavirus disease (COVID-19) Pandemic

41. Wrapp, D. et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science (*2020*).* doi:10.1126/science.abb2507

42. Letko, M., Marzi, A. & Munster, V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat. Microbiol. (*2020*).* doi:10.1038/s41564-020-0688-y

43. Wan, Y., Shang, J., Graham, R., Baric, R. S. & Li, F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. *J Virol*, *JVI.00127-00120*, doi: 10.1128/JVI.00127-20 *(*2020*).*

44. Andersen, K.G., Rambaut, A., Lipkin, W.I. *et al.* The proximal origin of SARS-CoV-2. *Nat Med* (2020). https://doi.org/10.1038/s41591-020-0820-9

45. Gui et al., 2017 M. Gui, W. Song, H. Zhou, J. Xu, S. Chen, Y. Xiang, X. Wang Cryo-electron microscopy structures of the SARS-CoV spike glycoprotein reveal a prerequisite conformational state for receptor binding Cell Res., 27 (2017), pp. 119-129

46. R.N. Kirchdoerfer R. N., C.A. Cottrell, N. Wang, J. Pallesen, H.M. Yassine, H.L. Turner, K.S. Corbett, B.S. Graham, J.S. McLellan, A.B. Ward Pre-fusion structure of a human coronavirus spike protein Nature, 531 (2016), pp. 118-121

47. Pallesen J., N. Wang, K.S. Corbett, D. Wrapp, R.N. Kirchdoerfer, H.L. Turner, C.A. Cottrell, M.M. Becker, L. Wang, W. Shi, *et al.* Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen Proc. Natl. Acad. Sci. USA, 114 (2017), pp. E7348-E7357

48. Song W., M. Gui, X. Wang, Y. Xiang Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2 PLoS Pathog., 14 (2018), p. e1007236

49. Walls A.C., M.A. Tortorici, B.J. Bosch, B. Frenz, P.J.M. Rottier, F. DiMaio, F.A. Rey, D. Veesler Cryo-electron microscopy structure of a coronavirus spike glycoprotein trime Nature, 531 (2016), pp. 114-117

50. Walls A.C., M.A. Tortorici, J. Snijder, X. Xiong, B.-J. Bosch, F.A. Rey, D. Veesler Tectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion Proc. Natl. Acad. Sci. USA, 114 (2017), pp. 11157-11162

51. Yuan Y., D. Cao, Y. Zhang, J. Ma, J. Qi, Q. Wang, G. Lu, Y. Wu, J. Yan, Y. Shi, *et al.*Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains Nat. Commun., 8 (2017), p. 15092

52. Belouzard S. , V.C. Chu, G.R. Whittaker Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites Proc. Natl. Acad. Sci. USA, 106 (2009), pp. 5871-5876

53. Heald-Sargent T , T. Gallagher Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence Viruses, 4 (2012), pp. 557-580

54. Millet J.K. , G.R. Whittaker Host cell entry of Middle East respiratory syndrome coronavirus after two-step, furin-mediated activation of the spike protein Proc. Natl. Acad. Sci. USA, 111 (2014), pp. 15214-15219

55. Millet J.K. , G.R. Whittaker Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis Virus Res., 202 (2015), pp. 120-134

56. ParkJ.E. , K. Li, A. Barlan, A.R. Fehr, S. Perlman, P.B. McCray Jr., T. Gallagher Proteolytic processing of Middle East respiratory syndrome coronavirus spikes expands virus tropism Proc. Natl. Acad. Sci. USA, 113 (2016), pp. 12262-12267

57. Walls A.C. , M.A. Tortorici, J. Snijder, X. Xiong, B J. Bosch, F.A. Rey, D. VeeslerTectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion Proc. Natl. Acad. Sci. USA, 114 (2017b), pp. 11157-11162

58. Tortorici M.A. , D. Veesler Structural insights into coronavirus entry Adv. Virus Res., 105 (2019), pp. 93-116

59. Hulswit R.J.G., Y. Lang, M.J.G. Bakkers, W. Li, Z. Li, A. Schouten, B. Ophorst, F.J.M. van Kuppeveld, G.J. Boons, B.J. Bosch, *et al.* Human coronaviruses OC43 and HKU1 bind to 9-***O***-acetylated sialic acids via a conserved receptor-binding site in spike protein domain A Proc. Natl. Acad. Sci. USA, 116 (2019), pp. 2681-2690

60. Tortorici M.A. , A.C. Walls, Y. Lang, C. Wang, Z. Li, D. Koerhuis, G.J. Boons, B.J. Bosch, F.A. Rey, R.J. de Groot, D. Veesler Structural basis for human coronavirus attachment to sialic acid receptors Nat. Struct. Mol. Biol., 26 (2019), pp. 481-489

61. Vlasak R., W. Luytjes, W. Spaan, P. Palese Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses Proc. Natl. Acad. Sci. USA, 85 (1988), pp. 4526-4529

62. Li W., R.J.G. Hulswit, I. Widjaja, V.S. Raj, R. McBride, W. Peng, W. Widagdo, M.A. Tortorici, B. van Dieren, Y. Lang, *et al.* Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein Proc. Natl. Acad. Sci. USA, 114 (2017), pp. E8508-E8517

63. Park Y.J., A.C. Walls, Z. Wang, M.M. Sauer, W. Li, M.A. Tortorici, B.J. Bosch, F. DiMaio, D. Veesler Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors Nat. Struct. Mol. Biol., 26 (2019), pp. 1151-1157

64. Lu G., Y. Hu, Q. Wang, J. Qi, F. Gao, Y. Li, Y. Zhang, W. Zhang, Y. Yuan, J. Bao, *et al* .Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26 Nature, 500 (2013), pp. 227-231

65. RajV.S., H. Mou, S.L. Smits, D.H. Dekkers, M.A. Müller, R. Dijkman, D. Muth, J.A. Demmers, A. Zaki, R.A. Fou chier, *et al.* Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC Nature, 495 (2013), pp. 251-254

66. Ge.X.Y. J.L. Li, X.L. Yang, A.A. Chmura, G. Zhu, J.H. Epstein, J.K. Mazet, B. Hu, W. Zhang, C. Peng, *et al.*Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor Nature, 503 (2013), pp. 535-538

67. Li F., W. Li, M. Farzan, S.C. Harrison Structure of SARS coronavirus spike receptor-binding domain complexed with receptor Science, 309 (2005 a), pp. 1864-1868

68. Li W., M.J. Moore, N. Vasilieva, J. Sui, S.K. Wong, M.A. Berne, M. Somasundaran, J.L. Sullivan, K. Luzuriaga, T.C. Greenough, *et al.*Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus Nature, 426 (2003), pp. 450-454

69. Yang X.L., B. Hu, B. Wang, M.N. Wang, Q. Zhang, W. Zhang, L.J. Wu, X.Y. Ge, Y.Z. Zhang, P. Daszak, *et al.* Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus J. Virol., 90 (2015), pp. 3253-3256

70. Desmyter J, Melnick JL, Rawls WE (October 1968). "Defectiveness of Interferon Production and of Rubella Virus Interference in a Line of African Green Monkey Kidney Cells (Vero)" *J. Virol*. **2** (10): 955–61. PMC 375423. PMID 4302013

71. Walls,A.C.;Park,Y.J.;Tortorici,M.A.;Wall,A.;McGuire,A.T.;Veesler,D.Structure,functionandantigenicity of the SARS-CoV-2 spike glycoprotein. bioRxiv 2020, 2020.02.19.956581.

72. Wong, M. C., Javornik Cregeen, S. J., Ajami, N. J. & Petrosino, J. F.bioRxiv https://doi.org/10.1101/2020.02.07.939207 (2020).

73. Liu, P., Chen, W. & Chen, J.-P.Viruses11, 979 (2019)

74. Poon, L. L. et al. Identification of a novel coronavirus in bats. *J. Virol*. **79**, 2001–2009 (2005).

75. Wang, N. et al. Serological evidence of bat SARS-related coronavirus infection in humans, China. *Virol. Sin*. 33, 104–107 (2018).

76. Owens, & Flores, & Di Serio, Francesco & Li, Shifang & Pallás, Vicente & Randles, John & Sano, & Vidalakis,. (2012). Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses.