UNC at Chapel HIll Request

[redacted] Mon, Aug 15, 2022 at 10:56 AM
Reply-To: [redacted]>
To: "cmssyc@gmail.com" <cmssyc@gmail.com>

Good morning Christine,

My name is [redacted]. I did this request https://unc.nextrequest.com/requests/22-258. I haven't received any documents they mentioned here. Maybe I'm missing something. I also send a request for NC Health Department. I haven't received anything on the side yet. Please guide me if you have time.

Thank you so much for all the work you have been doing. I will forever be grateful.

Sincerely,
[redacted]

UNC at Chapel HIll Request

[redacted] Sun, Sep 11, 2022 at 10:19 AM
Reply-To: [redacted]
To: Christine Massey <cmssyc@gmail.com>

Yes, this is what I got from them. Please guide me how to respond. I don't consider it answering the question.

Thank you so much for asking!

I kept saying I needed to have a good response before sharing with you. Not a good idea :)

https://unc.nextrequest.com/documents/14900185

H [redacted]

Sep 11, 2022, 10:24 AM (7 days ago)

to me

Just in case the link doesn't work .

Sent with Proton Mail secure email.

------- Original Message -------

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SARS-CoV-2: Combating Coronavirus Emergence

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https://doi.org/10.1016/j.immuni.2020.04.016

The emergence and rapid global spread of SARS-CoV-2 mark the third such identification of a novel coronavirus capable of causing severe, potentially fatal disease in humans in the 21st century. As noted by Andersen et al. (*Nature Medicine*), the sequencing of proximal zoonotic ancestors to SARS-CoV-2 has aided in the identification of alleles that may contribute to the virus' virulence in humans.

Three novel coronaviruses that are capable of causing severe disease have emerged in human populations in the 21st century. The 2003 severe acute respiratory coronavirus (SARS-CoV) and the 2012 Middle East respiratory coronavirus (MERS-CoV) foreshadowed the emergence potential of zoonotic coronaviruses. In December 2019, a strain of coronavirus that was 22% different from the 2003 SARS-CoV, later named severe acute respiratory syndrome strain 2 (SARS-CoV-2), emerged in Wuhan, China, and the resulting pandemic has caused over 3.2 million confirmed infections and over 225,000 deaths in nearly 5 months (as of April 29, 2020). The advent of high-throughput sequencing technologies has simplified the tracking of viral sequence diversity and evolution in both human and animal populations. Metagenomic surveillance of bat populations in areas near population centers in China has led to the identification of numerous civet and bat coronavirus strains closely related to the 2003 SARS-CoV. Moreover, strains like BatCoV-RaTG13 and pangolin GD/P2S1 share 96.2% and <90%, respectively, genome identity with SARS-CoV-2 (Lam et al., 2020; Zhang et al., 2020; Zhou et al., 2020) (Figure 1). Recently, Andersen et al. (2020) outlined the two most notable genetic features of SARS-CoV-2 that likely contribute to its virulence in humans: (1) a receptor-binding domain (RBD) that is optimized for binding to the human angiotensin-converting enzyme 2 (hACE2) molecule as the viral receptor and (2) the presence of a polybasic (furin) cleavage site at the S1-S2 boundary in the spike protein. The authors describe how these features contribute to virulence in other betacoronaviruses and then sketch two possibilities by which they may, through natural selection processes, have arisen in the coronavirus currently infecting humans worldwide.

Of the 14 residues of the RBD of 2003 SARS-CoV known to interact with hACE2 (Li et al., 2005), six residues are more critical for RBD-hACE2 binding and are host range determinants for SARS-CoV-like viruses (Wan et al., 2020). Interestingly, SARS-CoV and SARS-CoV-2 differ at 8/14 of these residues, including 5/6 critical interacting residues, and *in vitro* and structural studies indicate that SARS-CoV-2 has affinity for ACE2 molecules with high homology to hACE2 (Wan et al., 2020). However, while computational analyses indicate that this interaction has high affinity, the RBD sequence is clearly different from those shown to be optimal for hACE2 binding, suggesting that this binding interface is a product of a natural selection process on hACE2 or a human-like animal ACE2.

The other distinctive genetic feature SARS-CoV-2 possesses that potentially mediates virulence in humans is a polybasic (i.e., furin) cleavage site at the S1-S2 junction in the spike amino acid sequence. This site allows cleavage by proteases such as furin and is another factor that can determine viral infectivity and host range (Nao et al., 2017). While such cleavage sites have not been detected in other lineage B betacoronaviruses, they have been identified in betacoronaviruses in lineages A and C (in HCoV-HKU1 and MERS-like CoVs, respectively). Moreover, the O-linked glycans likely associated with the polybasic site may alter immunogenicity in response to herd immunity within natural animal hosts, which is likely not necessary in naive human populations (Bagdonaite and Wandall, 2018). Thus, the functional significance of the polybasic cleavage site awaits characterization.

In light of social media speculation about possible laboratory manipulation and deliberate and/or accidental release of SARS-CoV-2, Andersen et al. theorize about the virus' probable origins, emphasizing that the available data argue overwhelmingly against any scientific misconduct or negligence (Andersen et al., 2020). As has been previously described, the SARS-CoV-genome contains over 1,200 nucleotide changes as compared with RaTG13, its closest relative. Moreover, the RaTG13 S glycoprotein is 97% identical at the amino acid level to the SARS-CoV-2 S glycoprotein (Figure 1), and it encodes an RBD that is not optimized for hACE2 interaction (Wan et al., 2020). Anderson cites these genetic and biological data as strong evidence against deliberate generation, and the arguments are compelling. It is noteworthy that many early COVID-19 cases had not visited the Huanan wet market, suggesting that either the index cases occurred earlier and were not identified or that these sites were not major sites of epidemic expansion. How, then, did the virus emerge? Anderson et al. cite multiple lines of strong evidence that argue, instead, in favor of various mechanisms of natural selection, either in an animal host before the virus was transmitted to humans or in humans after the zoonotic transmission event(s). These possibilities will be reviewed below. Nevertheless, speculation about accidental laboratory escape will likely persist, given the large collections of bat virome samples stored in labs in the Wuhan Institute of Virology, the facility's proximity to the early outbreak, and the operating procedures at the facility

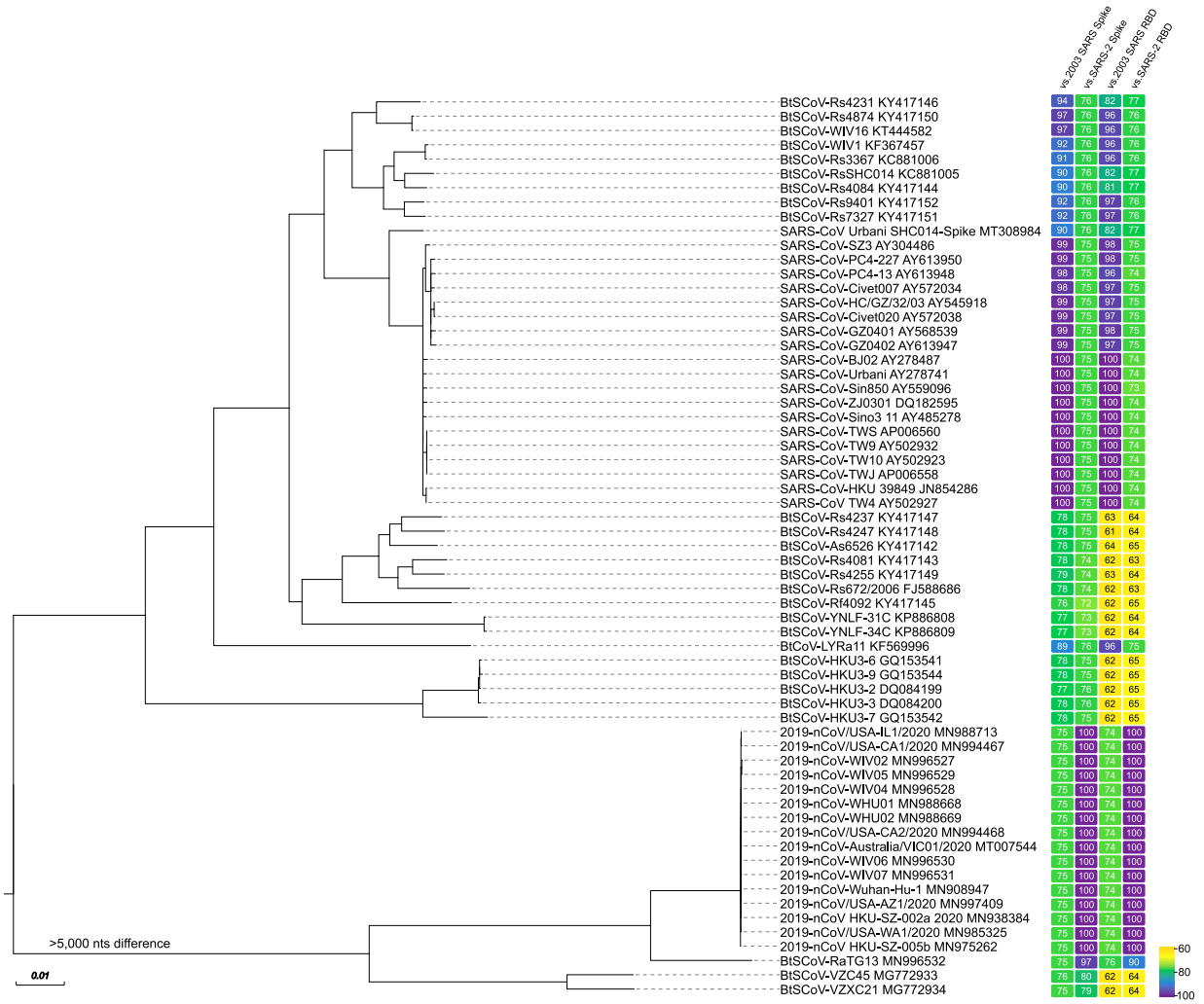

Figure 1. Genome Phylogeny and Spike and Receptor-Binding Domain Identity of Representative Group 2b Betacoronaviruses

The genome, spike, and RBD sequences of selected group 2b betacoronaviruses were aligned and phylogenetically compared. Sequences were aligned using free end gaps with the Blosum62 cost matrix in Geneious Prime. The phylogenetic tree was constructed from the multiple genome sequence alignment using the neighbor-joining method based on 100 replicates, also in Geneious Prime. The GenBank accession number follows each sequence name. Spike and RBD amino acid sequence identities from their respective alignments are represented by color-coded boxes to the right of each tree position, with colors ranging from yellow (~60% similarity) to purple (~100% similarity), shown in the scale in the lower right. Identities are represented as versus 2003 SARS-CoV spike (listed in figure as "2003 SARS Spike"), versus SARS-CoV-2 spike ("SARS-2 Spike"), versus 2003 SARS-CoV RBD ("2003 SARS RBD"), and versus SARS-CoV-2 RBD ("SARS-2 RBD"). The phylograms and alignments were exported from Geneious and then rendered for publication using EvolView (www.evolgenius.info) and Adobe Illustrator CC 2020.

(Zeng et al., 2016). Transparency and open scientific investigation will be essential to resolve this issue, noting that forensic evidence of natural escape is currently lacking, and other explanations remain reasonable.

Given the high correlation of many, but not all, of the early cases of COVID-19 disease in Wuhan with the Huanan wet market, it is possible that an animal reservoir of the virus was present at that location, and genome evolution analyses have suggested an earlier time of origin (Zhang et al., 2020). This scenario would have allowed for the establishment of earlier human-to-human transmission networks independent of the open market. The BtCoV-RaTG13 virus is the closest currently characterized relative to SARS-CoV-2, and it encodes 7/14 changes in the S glycoprotein RBD. More distantly related coronavirus genome sequences have also been identified in illegally imported Malayan pangolins (Lam et al., 2020), and while these strains encode 8/14 changes in the RBD interface residues, they do retain 6/6 of the most critical ACE2-interacting RBD residues with SARS-CoV-2 (Lam et al., 2020; Zhang et al., 2020). The presence of highly related viral sequences in diverse species argues strongly for natural selection being the major driving force for the optimization of the SARS-CoV-2 spike RBD among these related viruses. While a more homologous zoonotic relative has yet to be identified that shares the polybasic site with SARS-CoV-2, the sheer diversity of coronavirus sequences that have been identified in bat populations in China and worldwide indicates that zoonotic reservoirs are drastically under-sampled and

under-characterized. Clearly, additional studies into the diversity of zoonotic coronavirus strains are essential for global public health preparedness, for the development of countermeasures, and to clarify the origins of SARS-CoV-2.

Anderson et al. also argue that it is possible that a progenitor coronavirus jumped to humans prior to acquiring its polybasic site and key hACE2 interaction residues, acquiring these features through undetected human-to-human transmission events prior to the first documented cases of COVID-19 disease that triggered human surveillance systems (Wu et al., 2020; Zhou et al., 2020). In support, antibodies targeting the group 2b SARS-like coronaviruses can be detected in people living and working near or in bat hibernacula in China, suggesting frequent exposures in a rural setting. As SARS-CoV-2 infections are frequently asymptomatic or mild, initial exposures would easily have allowed for extended silent transmission events in rural settings prior to the emergence of a strain that could support sustained human-to-human transmission, especially when brought into an urban setting.

As emphasized by the authors, retroactive mapping of the paths of emergence of human pathogens is critical, especially in light of the global emergency fomented by the current pandemic. The presence of abundant sources of coronaviruses in zoonotic populations and the continuing and advancing encroachment of humans into animal habitats argue that emergence events will only become more common in future years. Indeed, prior to 2003, only two human coronaviruses were known: HCoV-OC43 and HCoV-229E, which cause mild, cold-like disease. After 2003, heightened surveillance retroactively identified two additional human coronaviruses, HCoV-NL63 and HCoV-HKU1. Nearly 10 years separated the documented emergence of SARS-CoV in 2003 and MERS-CoV in 2012, and just under 8 years have now separated the emergences of MERS-CoV and SARS-CoV-2. These patterns suggest that the global ecology has shifted and now favors the continued emergence of zoonotic coronaviruses, resulting in micro-outbreaks, continued low-level epidemics, or global pandemics.

In summary, Andersen et al. have outlined many of the key elements of the SARS-CoV-2 spike protein that could be mediating its extraordinary global expansion and summarize how the virus may have emerged from zoonotic populations (Andersen et al., 2020). The authors do not discuss the potential role for other less defined virulence determinants in the spike protein that alter host signaling networks and cytokine levels that may be associated with disease or transmission frequency. The virus, which is similar to yet distinct from the two previous zoonotic coronaviruses from the 21st century, SARS-CoV and MERS-CoV, marks the third emergence of a coronavirus that is capable of causing severe disease within the last 20 years. Novel bat coronaviruses have also emerged in swine populations in the past few years. As the pace of coronavirus emergence appears to be accelerating, these data not only underscore a common event in nature but also emphasize the urgency to develop vaccines and therapeutics with broad efficacy. Thus, studies characterizing the SARS-CoV-2 neutralizing epitopes and identifying broadly cross-neutralizing epitopes are clear priorities for immunotherapeutic and vaccine countermeasure design. This work should be performed with due caution, ensuring that putative enhancing epitopes are likewise identified and avoided in the course of vaccine design to minimize the risk of potentiating disease. Additionally, T cell epitopes should be identified across outbred populations to determine the key correlates of protective immunity. A key priority in combating the current pandemic and constructing readiness programs for future emergence events is the development of broadly effective medical countermeasures and therapeutics that can be stockpiled as insurance against future viral emergence events to prevent the human loss and economic and social catastrophe of global pandemics.

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The species *Severe acute respiratory syndrome-related coronavirus*: classifying 2019-nCoV and naming it SARS-CoV-2

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses*

The present outbreak of a coronavirus-associated acute respiratory disease called coronavirus disease 19 (COVID-19) is the third documented spillover of an animal coronavirus to humans in only two decades that has resulted in a major epidemic. The *Coronaviridae* Study Group (CSG) of the International Committee on Taxonomy of Viruses, which is responsible for developing the classification of viruses and taxon nomenclature of the family *Coronaviridae*, has assessed the placement of the human pathogen, tentatively named 2019-nCoV, within the *Coronaviridae*. Based on phylogeny, taxonomy and established practice, the CSG recognizes this virus as forming a sister clade to the prototype human and bat severe acute respiratory syndrome coronaviruses (SARS-CoVs) of the species *Severe acute respiratory syndrome-related coronavirus*, and designates it as SARS-CoV-2. In order to facilitate communication, the CSG proposes to use the following naming convention for individual isolates: SARS-CoV-2/host/location/isolate/date. While the full spectrum of clinical manifestations associated with SARS-CoV-2 infections in humans remains to be determined, the independent zoonotic transmission of SARS-CoV and SARS-CoV-2 highlights the need for studying viruses at the species level to complement research focused on individual pathogenic viruses of immediate significance. This will improve our understanding of virus–host interactions in an ever-changing environment and enhance our preparedness for future outbreaks.

Upon a viral outbreak, it is important to rapidly establish whether the outbreak is caused by a new or a previously known virus (Box 1), as this helps decide which approaches and actions are most appropriate to detect the causative agent, control its transmission and limit potential consequences of the epidemic. The assessment of virus novelty also has implications for virus naming and, on a different timescale, helps to define research priorities in virology and public health.

For many human virus infections such as influenza virus[1] or norovirus[2] infections, well-established and internationally approved methods, standards and procedures are in place to identify and name the causative agents of these infections and report this information promptly to public health authorities and the general public. In outbreaks involving newly emerged viruses, the situation may be different, and appropriate procedures to deal with these viruses need to be established or refined with high priority.

Here, we present an assessment of the genetic relatedness of the newly identified human coronavirus[3], provisionally named 2019-nCoV, to known coronaviruses, and detail the basis for (re)naming this virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which will be used hereafter. Given the public interest in naming newly emerging viruses and the diseases caused by these viruses in humans, we will give a brief introduction to virus discovery and classification — specifically the virus species concept — and the roles of different bodies, such as the World Health Organization (WHO) and the International Committee on Taxonomy of Viruses (ICTV), in this process. We hope this will help readers to better understand the scientific approach we have taken to arrive at this name, and we will also discuss implications of this analysis and naming decision.

Classifying and naming viruses and virus species

Defining the novelty of viruses is one of the topics that virus classification deals with. The classification of RNA viruses needs to consider their inherent genetic variability, which often results in two or more viruses with non-identical but similar genome sequences being regarded as variants of the same virus. This immediately poses the question of how much difference to an existing group is large enough to recognize the candidate virus as a member of a new, distinct group. This question is answered in best practice by evaluating the degree of relatedness of the candidate virus to previously identified viruses infecting 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. This is formally addressed in the framework of the official classification of virus taxonomy and is overseen and coordinated by the ICTV[4]. Viruses are clustered in taxa in a hierarchical scheme of ranks in which the species represents the lowest and most populous rank containing the least diverged groups (taxa) of viruses (Box 2). The ICTV maintains a Study Group for each virus family. The Study Groups are responsible for assigning viruses to virus species and taxa of higher ranks, such as subgenera, genera and subfamilies. In this context they play an important role in advancing the virus species concept and highlighting its significance[5].

Virus nomenclature is a formal system of names used to label viruses and taxa. The fact that there are names for nearly all viruses within a species is due to the historical perception of viruses as causative agents of specific diseases in specific hosts, and to the way we usually catalogue and classify newly discovered viruses, which increasingly includes viruses that have not been linked to any known disease in their respective hosts (Box 1). The WHO, an agency of the United Nations, coordinates international public health activities aimed at combating, containing and mitigating the consequences of communicable diseases—including major virus epidemics—and is responsible for naming disease(s) caused by newly emerging human viruses. In doing so, the WHO often takes the traditional approach of linking names of specific diseases to viruses (Box 1) and

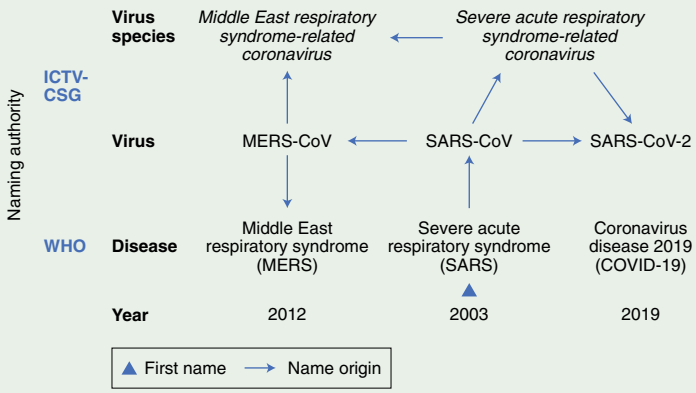
*A list of authors and their affiliations appears at the end of the paper.

Box 1 | Virus discovery and naming: from disease-based to phenotype-free

Understanding the cause of a specific disease that spreads among individuals of the same host species (infectivity) was the major driving force for the discovery of the first virus in plants, and subsequently many others in all forms of life, including humans. Historically, the range of diseases and hosts that specific viruses are associated with have been the two key characteristics used to define viruses, given that they are invisible to the naked eye[46]. Viral phenotypic features include those that, like a disease, are predominantly shaped by virus–host interactions including transmission rate or immune correlates of protection, and others that are largely virus-specific, such as the architecture of virus particles. These features are of critical importance to control, and respond to medically and economically important viruses — especially during outbreaks of severe disease — and dominate the general perception of viruses.

However, the host of a given virus may be uncertain, and virus pathogenicity remains unknown for a major (and fast-growing) proportion of viruses, including many coronaviruses discovered in metagenomics studies using next-generation sequencing technology of environmental samples[47,48]. These studies have identified huge numbers of viruses that circulate in nature and have never been characterized at the phenotypic level. Thus, the genome sequence is the only characteristic that is known for the vast majority of viruses, and needs to be used in defining specific viruses. In this framework, a virus is defined by a genome sequence that is capable of autonomous replication inside cells and dissemination between cells or organisms under appropriate conditions. It may or may not be harmful to its natural host. Experimental studies may be performed for a fraction of known viruses, while computational comparative genomics is used to classify (and deduce characteristics of) all viruses. Accordingly, virus naming is not necessarily connected to disease but rather informed by other characteristics.

In view of the above advancements and when confronted with the question of whether the virus name for the newly identified human virus should be linked to the (incompletely defined) disease that this virus causes, or rather be established independently from the virus phenotype, the CSG decided to follow a phylogeny-based line of reasoning to name this virus whose ontogeny can be traced in the figure in Box 1.



History of coronavirus naming during the three zoonotic outbreaks in relation to virus taxonomy and diseases caused by these viruses. According to the current international classification of diseases[49], MERS and SARS are classified as 1D64 and 1D65, respectively.

assessing virus novelty by an apparent failure to detect the causative agent using established diagnostic assays.

Apart from disease, geography and the organism from which a given virus was isolated also dominate the nomenclature, occasionally engraving connections that may be accidental (rather than typical) or even stigmatizing, which should be avoided. Establishing a universal nomenclature for viruses was one of the major tasks of the ICTV when it was founded more than 50 years ago[4]. When the species rank was established in the taxonomy of viruses[6], ICTV's responsibility for naming viruses was shifted to naming and establishing species. ICTV Study Groups may also be involved in virus naming on a case-by-case basis as an extension of their official remit, as well as using the special expertise of their members. As virus species names are often very similar to the name of the founding member of the respective species, they are frequently confused in the literature with names of individual viruses in this species. The species name is italicized, starts with a capital letter and should not be spelled in an abbreviated form[7]; hence the species name *Severe acute respiratory syndrome-related coronavirus*. In contrast, this convention does not apply to virus names, hence severe acute respiratory syndrome coronavirus, or SARS-CoV, as it is widely known.

Defining the place of SARS-CoV-2 within the *Coronaviridae*

Researchers studying coronaviruses—a family of enveloped positive-strand RNA viruses infecting vertebrates[8]—have been confronted several times with the need to define whether a newly emerged virus causing a severe or even life-threatening disease in humans belongs to an existing or a new (yet-to-be-established) species. This happened with SARS[9–12] and with Middle East respiratory syndrome (MERS)[13,14] a few years later. Each time, the virus was placed in the taxonomy using information derived from a sequence-based family classification[15,16].

The current classification of coronaviruses recognizes 39 species in 27 subgenera, five genera and two subfamilies that belong to the family *Coronaviridae*, suborder *Cornidovirineae*, order *Nidovirales* and realm *Riboviria*[17–19] (Fig. 1). The family classification and taxonomy are developed by the *Coronaviridae* Study Group (CSG), a working group of the ICTV[20]. The CSG is responsible for assessing

Box 2 | Identifying viral species

The terms strain and isolate are commonly used to refer to virus variants, although there are different opinions as to which term should be used in a specific context. If a candidate virus clusters within a known group of isolates, it is a variant of this group and may be considered as belonging to this known virus group. In contrast, if the candidate virus is outside of known groups and its distances to viruses in these groups are comparable to those observed between viruses of different groups (intergroup distances), the candidate virus is distinct and can be considered novel.

This evaluation is usually conducted in silico using phylogenetic analysis, which may be complicated by uneven rates of evolution that vary across different virus lineages and genomic sites due to mutation, including the exchange of genome regions between closely related viruses (homologous recombination). However, given that the current sampling of viruses is small and highly biased toward viruses of significant medical and economic interest, group composition varies tremendously among different viruses, making decisions on virus novelty group-specific and dependent on the choice of the criteria selected for this assessment.

These challenges are addressed in the framework of virus taxonomy, which partitions genomic variation above strain or isolate level and develops a unique taxon nomenclature under the supervision of the ICTV[4,5]. To decide on whether a virus represents a new species—that is, the least diverged (and most populated) group of viruses—taxonomists use the results of different analyses. Taxonomical classification is hierarchical, using nested groups (taxa) that populate different levels (ranks) of classification. Taxa of different ranks differ in their intra-taxon pairwise divergence, which increases from the smallest at the species rank to the largest at the realm rank[30]. They may also be distinguished by taxon-specific markers that characterize natural groupings. Only the species and genus ranks need to be specified to classify a new virus; filling other ranks is optional. If a virus prototypes a new species, it will be regarded as taxonomically novel. If (within this framework) a virus crosses a host barrier and acquires novel properties, its classification will not change (that is, it remains part of the original species) even if the virus establishes a permanent circulation in the new host, which likely happened with coronaviruses of the four species that circulate in humans and display seasonal peaks (reviewed in ref. [50]). Importantly, the criteria used to define a viral species in one virus family such as *Coronaviridae* may not be applicable to another family such as *Retroviridae*, and vice versa, since Study Groups are independent in their approach to virus classification.

Box 3 | Classifying coronaviruses

Initially, the classification of coronaviruses was largely based on serological (cross-) reactivities to the viral spike protein, but is now based on comparative sequence analyses of replicative proteins. The choice of proteins and the methods used to analyse them have gradually evolved since the start of this century[20,28,29,51]. The CSG currently analyses 3CLpro, NiRAN, RdRp, ZBD and HEL1 (ref. [52]) (Fig. 2a), two domains less than previously used in the analyses conducted between 2009 and 2015 (refs. [16,18]). According to our current knowledge, these five essential domains are the only ones conserved in all viruses of the order *Nidovirales*[52]. They are thus used for the classification by all ICTV nidovirus study groups (coordinated by the NSG).

Since 2011, the classification of coronaviruses and other nidoviruses has been assisted by the DivErsity pArtitioning by hieRarchical Clustering (DEmARC) software, which defines taxa and ranks[23,24]. Importantly, the involvement of all coronavirus genome sequences available at the time of analysis allows family-wide designations of demarcation criteria for all ranks, including species, regardless of the taxa sampling size, be it a single or hundreds of virus(es). DEmARC delineates monophyletic clusters (taxa) of viruses using weighted linkage clustering in the PPD space and according to the classification of ranks defined through clustering cost (CC) minima presented as PPD thresholds (PPD accounts for multiple substitutions at all sequence positions and thus may exceed 1.0, which is the limit for conventional pair-wise distances (PDs)). In the DEmARC framework, the persistence of thresholds in the face of increasing virus sampling is interpreted to reflect biological forces and environmental factors[21]. Homologous recombination, which is common in coronaviruses[53–55], is believed to be restricted in genome regions encoding the most essential proteins, such as those used for classification, and to members of the same virus species. This restriction promotes intra-species diversity and contributes to inter-species separation. To facilitate the use of rank thresholds outside of the DEmARC framework, they are converted into PD and expressed as a percentage, which researchers commonly use to arrive at a tentative assignment of a given virus within the coronavirus taxonomy following conventional phylogenetic analysis of selected viruses.

the place of new viruses through their relation to known viruses in established taxa, including placements relating to the species *Severe acute respiratorysyndrome-related coronavirus*. In the classification of nidoviruses, species are considered biological entities demarcated by a genetics-based method[21], while generally virus species are perceived as man-made constructs[22]. To appreciate the difference between a nidoviral species and the viruses grouped therein, it may be instructive to look at their relationship in the context of the full taxonomy structure of several coronaviruses. Although these viruses were isolated at different times and locations from different human and animal hosts (with and without causing clinical disease), they all belong to the species *Severe acute respiratorysyndrome-related coronavirus*, and their relationship parallels that between human individuals and the species *Homo sapiens* (Fig. 1).

Even without knowing anything about the species concept, every human recognizes another human as a member of the same species. However, for assigning individual living organisms to most other species, specialized knowledge and tools for assessing inter-individual differences are required. The CSG uses a computational framework of comparative genomics[23], which is shared by several ICTV Study Groups responsible for the classification and nomenclature of the order *Nidovirales* and coordinated by the ICTV *Nidovirales* Study Group (NSG)[24] (Box 3). 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 identify thresholds on pair-wise patristic distances (PPDs) that demarcate virus clusters at different ranks.

Consistent with previous reports, SARS-CoV-2 clusters with SARS-CoVs in trees of the species *Severe acute respiratory syndrome-related coronavirus* (Fig. 2b) and the genus *Betacoronavirus* (Fig. 2c)[25–27]. Distance estimates between SARS-CoV-2 and the most closely related coronaviruses vary among different studies depending on the choice of measure (nucleotide or amino acid) and genome region. Accordingly, there is no agreement yet on the exact taxonomic position of SARS-CoV-2 within the subgenus *Sarbecovirus*. When we included SARS-CoV-2 in the dataset used for the most recent update (May 2019) of the coronavirus taxonomy currently being considered by ICTV[19], which includes 2,505 coronaviruses,

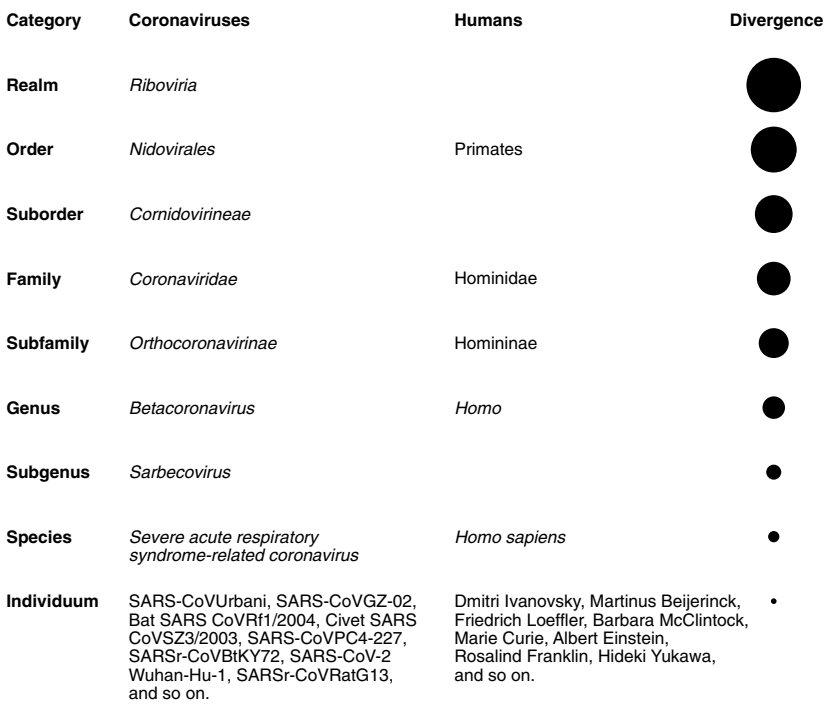


Fig. 1 | Taxonomy of selected coronaviruses. Shown is the full taxonomy of selected coronaviruses in comparison with the taxonomy of humans (the founders of virology and other eminent scientists represent individual human beings for the sake of this comparison), which is given only for categories (ranks) that are shared with the virus taxonomy. Note that these two taxonomies were independently developed using completely different criteria. Although no equivalence is implied, the species of coronaviruses is interpreted sensu stricto as accepted for the species of humans.

the species composition was not affected and the virus was assigned to the species *Severe acute respiratory syndrome-related coronavirus*, as detailed in Box 4.

With respect to novelty, SARS-CoV-2 differs from the two other zoonotic coronaviruses, SARS-CoV and MERS-CoV, introduced to humans earlier in the twenty-first century. Previously, the CSG established that each of these two viruses prototype a new species in a new informal subgroup of the genus *Betacoronavirus*[15,16]. These two informal subgroups were recently recognized as subgenera *Sarbecovirus* and *Merbecovirus*[18,28,29] when the subgenus rank was established in the virus taxonomy[30]. Being the first identified representatives of a new species, unique names were introduced for the two viruses and their taxa in line with the common practice and state of virus taxonomy at the respective times of isolation. The situation with SARS-CoV-2 is fundamentally different because this virus is assigned to an existing species that contains hundreds of known viruses predominantly isolated from humans and diverse bats. All these viruses have names derived from SARS-CoV, although only the human isolates collected during the 2002–2003 outbreak have been confirmed to cause SARS in infected individuals. Thus, the reference to SARS in all these virus names (combined with the use of specific prefixes, suffixes and/or genome sequence IDs in public databases) acknowledges the phylogenetic (rather than clinical disease-based) grouping of the respective virus with the prototypic virus in that species (SARS-CoV). The CSG chose the name SARS-CoV-2 based on the established practice for naming viruses in this species and the relatively distant relationship of this virus to the prototype SARS-CoV in a species tree and the distance space (Fig. 2b and the figure in Box 4).

The available yet limited epidemiological and clinical data for SARS-CoV-2 suggest that the disease spectrum and transmission efficiency of this virus[31–35] differ from those reported for SARS-CoV[9]. To accommodate the wide spectrum of clinical presentations and outcomes of infections caused by SARS-CoV-2 (ranging from asymptomatic to severe or even fatal in some cases)[31], the WHO recently introduced a rather unspecific name (coronavirus disease 19, also known as COVID-19 (ref. [36])) to denote this disease. 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 (for example, the WHO guidelines for SARS-CoV-2 (ref. [37])). By uncoupling the naming conventions used for coronaviruses and the diseases that some of them cause in humans and animals, we wish to support the WHO in its efforts to establish disease names in the most appropriate way (for further information, see the WHO's guidelines for disease naming[38]). The further advancement of naming conventions is also important because the ongoing discovery of new human and animal viruses by next-generation sequencing technologies can be expected to produce an increasing number of viruses that do not (easily) fit the virus–disease model that was widely used in the pre-genomic era (Box 1). Having now established different names for the causative virus (SARS-CoV-2) and the disease (COVID-19), the CSG hopes that this will raise awareness in both the general public and public health authorities regarding the difference between these two entities. The CSG promotes this clear distinction because it will help improve the outbreak management and also reduces the risk of confusing virus and disease, as has been the case over many years with SARS-CoV (the virus) and SARS (the disease).

To facilitate good practice and scientific exchange, the CSG recommends that researchers describing new viruses (that is, isolates) in this species adopt a standardized format for public databases and publications that closely resembles the formats used for isolates of avian coronaviruses[39], filoviruses[40] and influenza virus[1]. The proposed naming convention includes a reference to the host organism that the virus was isolated from, the place of isolation (geographic location), an isolate or strain number, and the time of isolation (year or more detailed) in the format virus/host/location/isolate/date; for

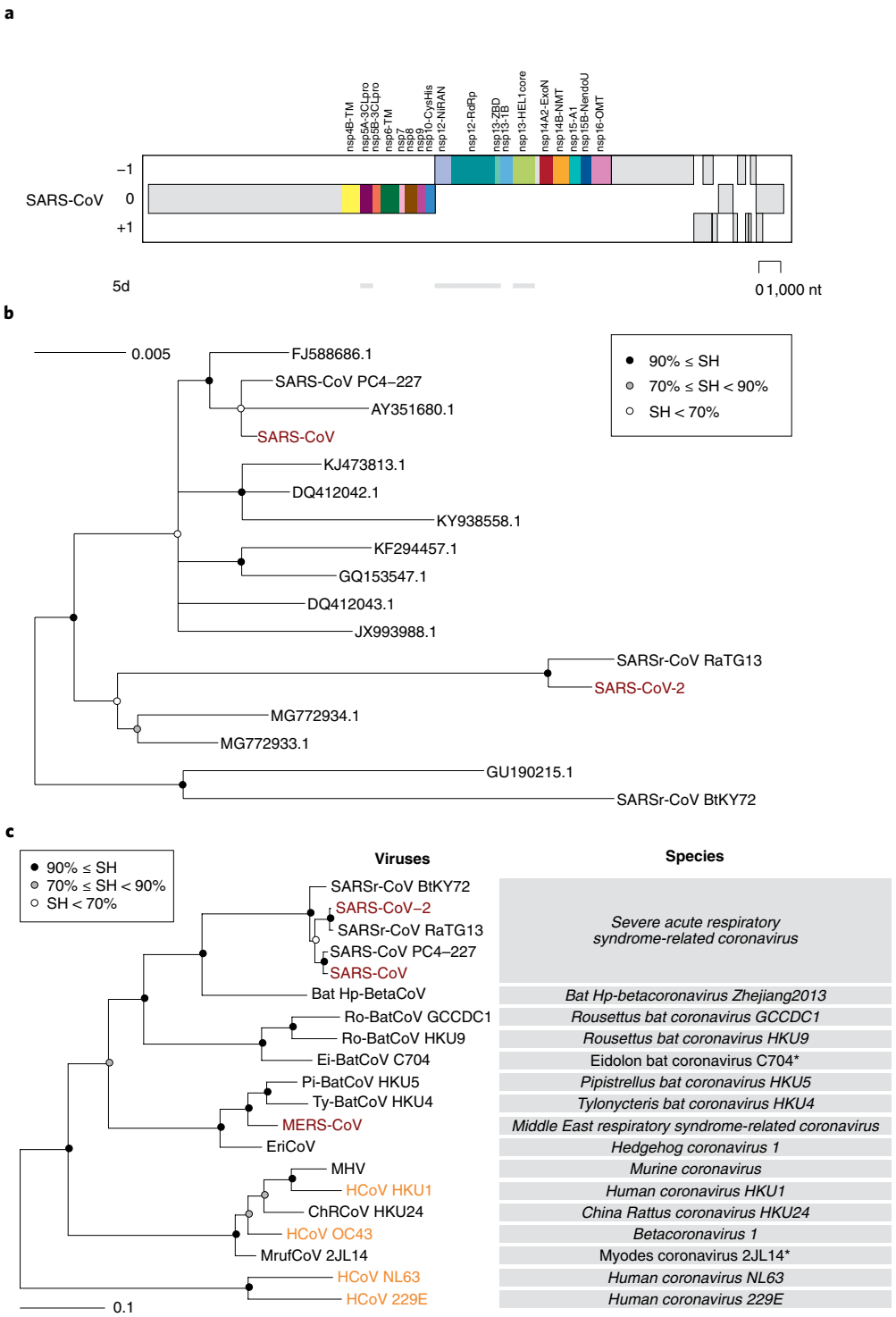
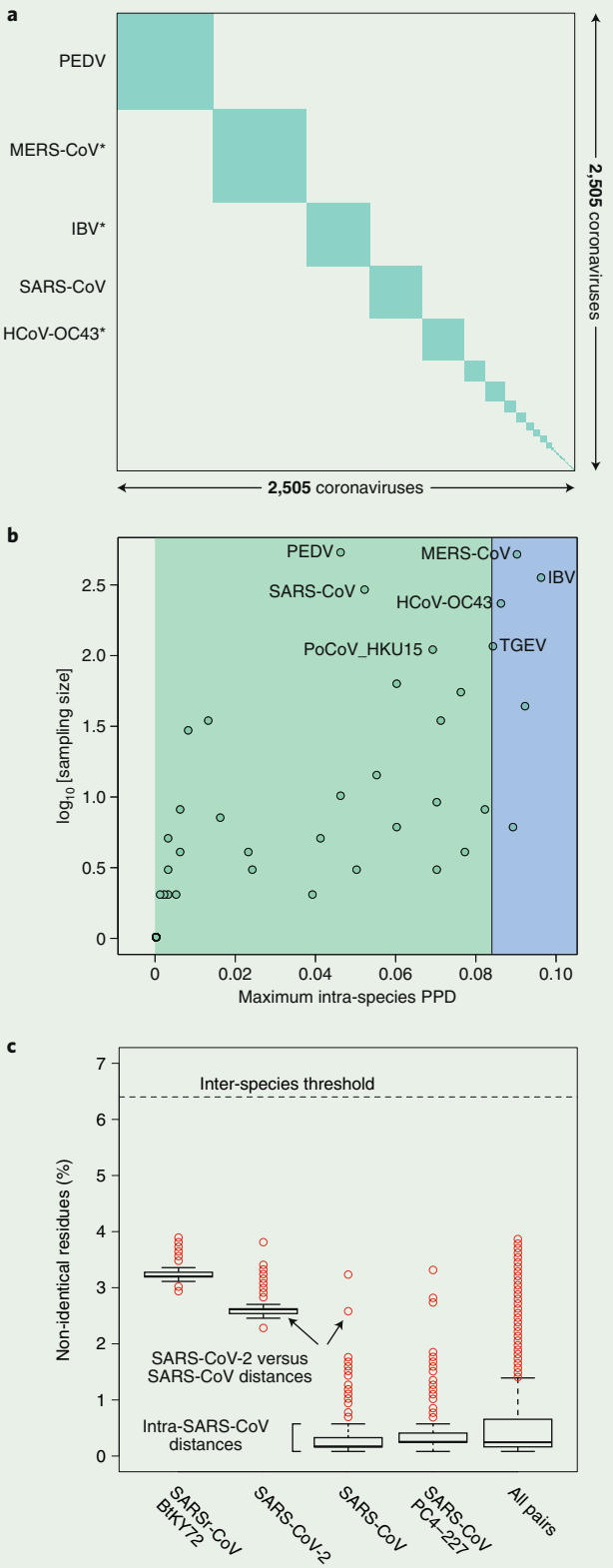


Fig. 2 | Phylogeny of coronaviruses. a, Concatenated multiple sequence alignments (MSAs) of the protein domain combination[44] used for phylogenetic and DEmARC analyses of the family *Coronaviridae*. Shown are the locations of the replicative domains conserved in the ordert *Nidovirales* in relation to several other ORF1a/b-encoded domains and other major ORFs in the SARS-CoV genome. 5d, 5 domains: nsp5A-3CLpro, two beta-barrel domains of the 3C-like protease; nsp12-NiRAN, nidovirus RdRp-associated nucleotidyltransferase; nsp12-RdRp, RNA-dependent RNA polymerase; nsp13-HEL1 core, superfamily 1 helicase with upstream Zn-binding domain (nsp13-ZBD); nt, nucleotide. **b**, The maximum-likelihood tree of SARS-CoV was reconstructed by IQ-TREE v.1.6.1 (ref. [45]) 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 (SH)-like approximate likelihood ratio test with 1,000 replicates. GenBank IDs for all viruses except four are shown; SARS-CoV, AY274119.3; SARS-CoV-2, MN908947.3; SARSr-CoV_BtKY72, KY352407.1; SARS-CoV_PC4-227, AY613950.1. **c**, Shown is an IQ-TREE maximum-likelihood tree of single virus representatives of thirteen species and five representatives of the species *Severe acute respiratory syndrome-related coronavirus* of the genus *Betacoronavirus*. The tree is rooted with HCoV-NL63 and HCoV-229E, representing two species of the genus *Alphacoronavirus*. Purple text highlights zoonotic viruses with varying pathogenicity in humans; orange text highlights common respiratory viruses that circulate in humans. Asterisks indicate two coronavirus species whose demarcations and names are pending approval from the ICTV and, thus, these names are not italicized.

Box 4 | Classifying SARS-CoV-2

The species demarcation threshold (also known as demarcation limit) in the family *Coronaviridae* is defined by viruses whose PPD(s) may cross the inter-species demarcation PPD threshold (threshold 'violators'). Due to their minute share of ~10^{-4} of the total number of all intra- and inter-species PPDs, these violators may not even be visually recognized in a conventional diagonal plot clustering viruses on a species basis (panel **a** of the figure in Box 4). Furthermore, they do not involve any virus of the species *Severe acute respiratory syndrome-related coronavirus*, as is evident from the analysis of maximal intraspecies PPDs of 2,505 viruses of all 49 coronavirus species (of which 39 are established and 10 are pending or tentative) (panel **b** of the figure in Box 4) and PDs of 256 viruses of this species (panel **c** of the figure in Box 4). Thus, the genomic variation of the known viruses of the species *Severe acute respiratory syndrome-related coronavirus* is smaller compared to that of other comparably well-sampled species—for example, those prototyped by MERS-CoV, human coronavirus OC43 (HCoV-OC43) and infectious bronchitis virus (IBV) (panel **b** of the figure in Box 4)—and this species is well separated from other known coronavirus species in the sequence space. Both of these characteristics facilitate the unambiguous assignment of SARS-CoV-2 to this species.

Intra-species PDs of SARS-CoV-2 belong to the top 25% of this species and also include the largest PD between SARS-CoV-2 and an African bat virus isolate (SARSr-CoV_BtKY72)[56] (panel **c** of the figure in Box 4), representing two basal lineages within the species *Severe acute respiratory syndrome-related coronavirus* that constitute very few known viruses (Fig. 2b,c). 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[57]. This clade structure is susceptible to homologous recombination, which is common in this species[44,58,59]; to formalize clade definition, it must be revisited after the sampling of viruses representing the deep branches has improved sufficiently. 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% versus 2.6%; panel **c** of the figure in Box 4). This small median PD of human SARS-CoVs also dominates the species-wide PD distribution (0.25%; panel **c** of the figure in Box 4).



Pairwise distance demarcation of species in the family *Coronaviridae*. a, Diagonal matrix of PPDs of 2,505 viruses clustered according to 49 coronavirus species, 39 established and 10 pending or tentative, and ordered from the most to least populous species, from left to right; green and white, PPDs smaller and larger than the inter-species threshold, 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; asterisks indicate species that include viruses whose intra-species PPDs crossed the inter-species threshold (threshold 'violators'). **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 represent intra-species and intra-subgenera PPD ranges. The vertical black line indicates the inter-species threshold. **c**, Shown are the PDs of non-identical residues (*y* axis) for four viruses representing three major phylogenetic lineages (clades) of the species *Severe acute respiratorysyndrome-related coronavirus* (panel **b**) and all pairs of the 256 viruses of this species ('all pairs'). The PD values were derived from pairwise distances in the MSA that were calculated using an identity matrix. Panels **a** and **b** were adopted from the DEmARC v.1.4 output.

example, SARS-CoV-2/human/Wuhan/X1/2019. This complete designation along with additional and important characteristics, such as pathogenic potential in humans or other hosts, should be included in the submission of each isolate genome sequence to public databases such as GenBank. In publications, this name could be further extended with a sequence database ID—for example, SARS-CoV-2/human/Wuhan/X1/2019_XYZ12345 (fictional example)—when first mentioned in the text. We believe that this format will provide critical metadata on the major characteristics of each particular virus isolate (genome sequence) required for subsequent epidemiological and other studies, as well as for control measures.

Expanding the focus from pathogens to virus species

Historically, public health and fundamental research have been focused on the detection, containment, treatment and analysis of viruses that are pathogenic to humans following their discovery (a reactive approach). Exploring and defining their biological characteristics in the context of the entire natural diversity as a species has never been a priority. The emergence of SARS-CoV-2 as a human pathogen in December 2019 may thus be perceived as completely independent from the SARS-CoV outbreak in 2002–2003. Although SARS-CoV-2 is indeed not a descendent of SARS-CoV (Fig. 2b), and the introduction of each of these viruses into humans was likely facilitated by independent unknown external factors, the two viruses are genetically so close to each other (Fig. 2c, panel **c** of the figure in Box 4) that their evolutionary histories and characteristics are mutually informative.

The currently known viruses of the species *Severe acute respiratory syndrome-related coronavirus* may be as (poorly) representative for this particular species as the few individuals that we selected to represent *H. sapiens* in Fig. 1. It is thus reasonable to assume that this biased knowledge of the natural diversity of the species *Severe acute respiratory syndrome-related coronavirus* limits our current understanding of fundamental aspects of the biology of this species and, as a consequence, our abilities to control zoonotic spillovers to humans. Future studies aimed at understanding the ecology of these viruses and advancing the accuracy and resolution of evolutionary analyses[41] would benefit greatly from adjusting our research and sampling strategies. This needs to include an expansion of our current research focus on human pathogens and their adaptation to specific hosts to other viruses in this species. To illustrate the great potential of species-wide studies, it may again be instructive to draw a parallel to *H. sapiens*, and specifically to the impressive advancements in personalized medicine in recent years. Results of extensive genetic analyses of large numbers of individuals representing diverse populations from all continents have been translated into clinical applications and greatly contribute to optimizing patient-specific diagnostics and therapy. They were instrumental in identifying reliable predictive markers for specific diseases as well as genomic sites that are under selection. It thus seems reasonable to expect that genome-based analyses with a comparable species coverage will be similarly insightful for coronaviruses. Also, additional diagnostic tools that target the entire species should be developed to complement existing tools optimized to detect individual pathogenic variants (a proactive approach). Technical solutions to this problem are already available; for example, in the context of multiplex PCR-based assays[42]. The costs for developing and applying (combined or separate) species- and virus-specific diagnostic tests in specific clinical and/or epidemiological settings may help to better appreciate the biological diversity and zoonotic potential of specific virus species and their members. Also, the further reduction of time required to identify the causative agents of novel virus infections will contribute to limiting the enormous social and economic consequences of large outbreaks. To advance such studies, innovative fundraising approaches may be required.

Although this Consensus Statement focuses on a single virus species, the issues raised apply to other species in the family and possibly beyond. A first step towards appreciation of this species and others would be for researchers, journals, databases and other relevant bodies to adopt proper referencing to the full taxonomy of coronaviruses under study, including explicit mentioning of the relevant virus species and the specific virus(es) within the species using the ICTV naming rules explained above. This naming convention is, regretfully, rarely observed in common practice, with mixing of virus and species names being frequently found in the literature (including by the authors of this Consensus Statement on several past occasions). The adoption of accurate virus-naming practices should be facilitated by the major revision of the virus species nomenclature that is currently being discussed by the ICTV and is being planned for implementation in the near future[43]. With this change in place, the CSG is resolved to address the existing significant overlap between virus and species names that complicates the appreciation and use of the species concept in its application to coronaviruses.

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Author contributions

S.C.B., R.S.B., C.D., R.J.D.G., A.E.G., B.L.H., B.W.N., S.P., L.L.M.P., I.S. and J.Z. are members of the CSG, chaired by J.Z.; R. J.D.G., A.E.G., C.L., B.W.N. and J.Z. are members of the NSG, chaired by A.E.G.; A.E.G. and J.Z. are members of the ICTV. A.E.G., A.A.G., C.L., A.M.L., D.P., D.V.S. and I.A.S. are members of the DEmARC team led by A.E.G. D.V.S. generated the classification of SARS-CoV-2 using a computational pipeline developed by A.A.G. and using software developed by the DEmARC team; the CSG considered and approved this classification, and subsequently debated and decided on the virus name. A.E.G. and J.Z. wrote the manuscript. A.E.G. and D.V.S. generated the figures. All authors reviewed the manuscript and approved its submission for publication.

Competing interests

The authors declare no competing interests.

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CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor*

Emergence of a Highly Fit SARS-CoV-2 Variant

Ralph S. Baric, Ph.D.

Sarbecoviruses have emerged twice in the 21st century, causing a worldwide epidemic and pandemic. The ongoing pandemic of coronavirus disease 2019 (Covid-19), the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused unprecedented disruption of human society. Since its emergence in December 2019, SARS-CoV-2 has spread worldwide, infecting more than 70 million persons and causing more than 1.6 million deaths as of early December 2020. Previous studies have clearly shown that epidemic and pandemic RNA virus spread may select for mutations that alter RNA virus pathogenesis, virulence, transmissibility, or a combination of these,[1] yet this process remains poorly studied among emerging coronaviruses in animals and humans.

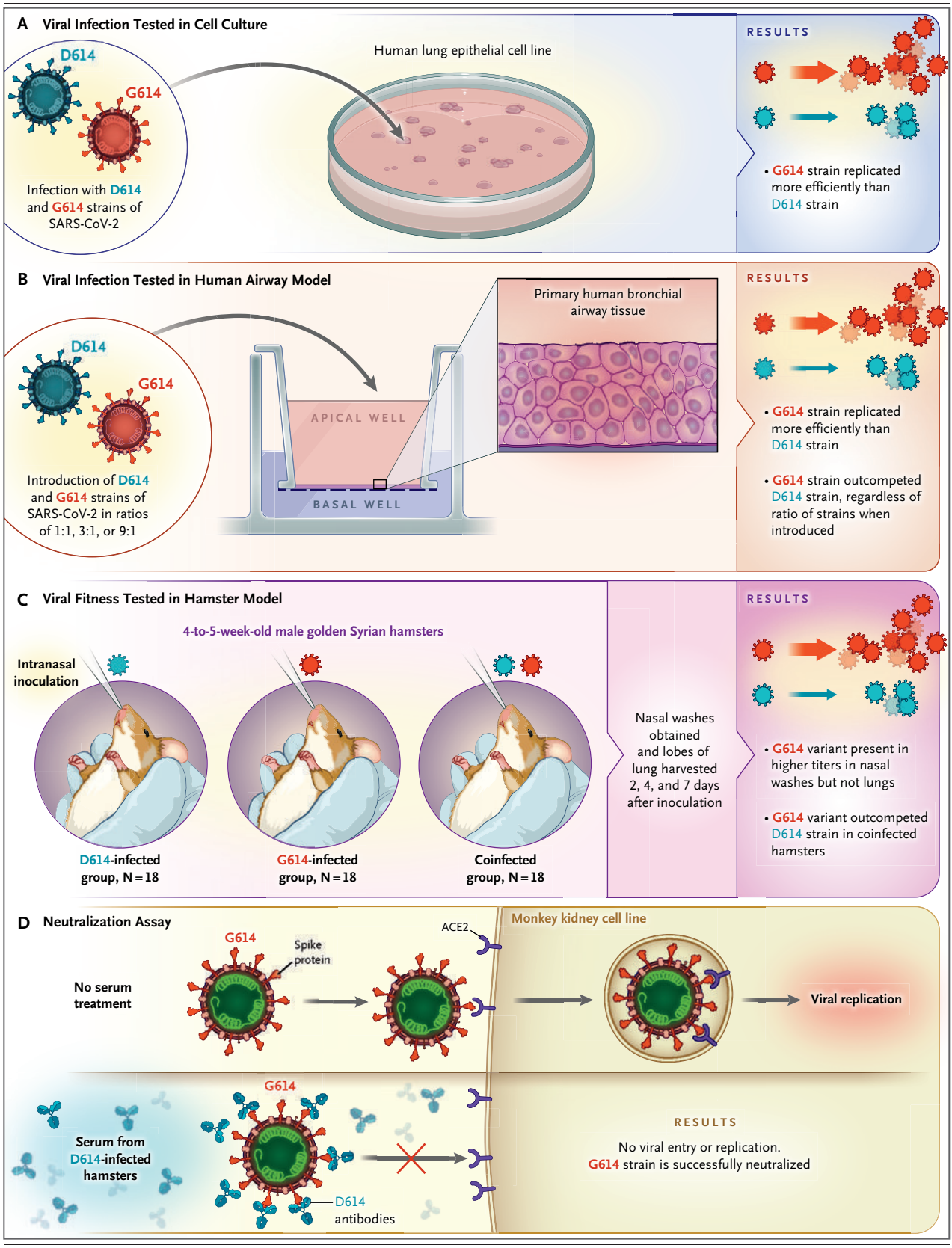
SARS-CoV-2 probably emerged from bats, and early strains identified in Wuhan, China, showed limited genetic diversity, which suggests that the virus may have been introduced from a single source.[2] Early zoonotic variants in the novel coronavirus SARS-CoV that emerged in 2003 affected the receptor-binding domain (RBD) of the spike protein and thereby enhanced virus docking and entry through the human angiotensin-converting–enzyme 2 (hACE2) receptor.[3] In contrast, the spike-protein RBD of early SARS-CoV-2 strains was shown to interact efficiently with hACE2 receptors early on.[2]

However, despite the presence of a CoV RNA proofreading activity that yields high replication fidelity, genetic epidemiologic investigations conducted in late February identified an emerging D614G mutation affecting the spike glycoprotein of SARS-CoV-2 strains from southern Europe; this variant has since spread rapidly and has become the most prevalent genotype worldwide.[4] Patients infected with D614G-associated SARS-CoV-2 are more likely to have higher viral loads in the upper respiratory tract than patients infected with virus strains without the mutation, but disease severity is not affected. Pseudotyped viruses with the G614 form of the SARS-CoV-2 spike protein have been reported to exhibit increased infectivity in continuous cell lines and increased sensitivity to neutralization. In addition, structural analyses have revealed that the RBD of the G614 form of the spike protein is more likely to assume an "open" conformation than the RBD of the ancestral D614 form, implying an improved ability to bind to the hACE2 receptor. However, published reports of isolation of the D614G substitution in an authentic SARS-CoV-2 recombinant live virus are lacking, as are investigations on the effects of the mutation on in vivo replication and pathogenesis.

In a recent study, Plante et al. used reverse genetics to recover isogenic recombinant SARS-CoV viruses encoding the D614G mutation.[5] The G614 variant replicated more efficiently than did the D614 variant in immortalized cells in culture and in primary human airway epithelial cells (Fig. 1A and 1B). Even at D614-to-G614 variant infection ratios of 1:1, 3:1, or 9:1, the contemporary

Figure 1 (facing page). Increased Infectivity of SARS-CoV-2 Bearing the Spike Protein D614G Substitution.

A study recently reported by Plante et al.[5] showed that a variant of SARS-CoV-2 carrying the spike protein D614G substitution results in increased virus infectivity and yield in human lung epithelial cells (Panel A), in primary human airway tissue (Panel B), and in the upper airway of hamsters (Panel C). These data suggest that the D614G mutation results in enhanced transmissibility. In addition, serum samples from D614-virus–infected hamsters can efficiently neutralize the G614 virus from infecting cells (Panel D), which suggests that SARS-CoV-2 vaccines, all of which are based on the D614 variant of the spike protein, will protect against G614 variants of the virus.



G614 strain outcompeted the ancestral D614 strain in primary human airway epithelial cells. The G614 variant also seemed to be more stable than the ancestral strain, which suggests that increased stability may be associated with increased infectivity, although additional investigations will be needed to confirm this finding.

In studies in hamsters infected with D614 or G614 variants, Plante et al. showed that the contemporary G614 variant replicated to higher titers in nasal-wash samples early after infection and outcompeted the ancestral D614 variant (Fig. 1C); these findings suggest increased fitness in a major upper airway compartment potentially associated with enhanced transmission. The SARS-CoV-2 G614 variant did not cause more severe disease than the ancestral strain in hamsters, a finding that supports current findings in humans. The Covid-19 vaccines that are currently being evaluated in clinical trials are based on the original D614 ancestral spike sequence; therefore, the authors used a panel of serum specimens to test whether the G614 variant is as sensitive to neutralization as the ancestral strain (Fig. 1D). Fortunately, the results showed that it is as sensitive to the serum specimens as the D614 strain and thus may allay fears that it could escape vaccine-elicited immunity.

Plante et al. have provided evidence of the genetic and molecular basis for enhanced fitness of the G614 variant over ancestral strains, providing strong support for its role in facilitating global spread. Unlike variants in the SARS-CoV 2003 epidemic strain, those in SARS-CoV-2 may point to new mechanisms that are associated with pandemic spread in human populations. In addition to showing the critical importance of blending genetic epidemiologic studies with empirical molecular virologic studies to understand pandemic virus evolution and spread, the findings raise critical questions regarding the future evolutionary trajectories of the SARS-CoV-2 G614 variant. These questions are especially important at a time when environmental pressures, such as expanding herd immunity, vaccine-induced immunity, antiviral therapies, and public health intervention strategies, may — through selective pressure — promote virus survival and escape. Will these selective pressures drive antigenic variation, promote virus stability and transmissibility, alter virus virulence and pathogenesis, or drive SARS-CoV-2 to extinction or into alternative hosts as reservoirs? Plante et al. articulate a critical need for proactive, rather than reactive, tracking of SARS-CoV-2 and other potential emerging coronaviruses.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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LETTERS

Edited by **Jennifer Sills**

Investigate the origins of COVID-19

On 30 December 2019, the Program for Monitoring Emerging Diseases notified the world about a pneumonia of unknown cause in Wuhan, China (*1*). Since then, scientists have made remarkable progress in understanding the causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), its transmission, pathogenesis, and mitigation by vaccines, therapeutics, and non-pharmaceutical interventions. Yet more investigation is still needed to determine the origin of the pandemic. Theories of accidental release from a lab and zoonotic spillover both remain viable. Knowing how COVID-19 emerged is critical for informing global strategies to mitigate the risk of future outbreaks.

In May 2020, the World Health Assembly requested that the World Health Organization (WHO) director-general work closely with partners to determine the origins of SARS-CoV-2 (*2*). In November, the Terms of Reference for a China–WHO joint study were released (*3*). The information, data, and samples for the study's first phase were collected and summarized by the Chinese half of the team; the rest of the team built on this analysis. Although there were no findings in clear support of either a natural spillover or a lab accident, the team assessed a zoonotic spillover from an intermediate host as "likely to very likely," and a laboratory incident as "extremely unlikely" [(*4*), p. 9]. Furthermore, the two theories were not given balanced consideration. Only 4 of the 313 pages of the report and its annexes addressed the possibility of a laboratory accident (*4*). Notably, WHO Director-General Tedros Ghebreyesus commented that the report's consideration of evidence supporting a laboratory accident was insufficient and offered to provide additional resources to fully evaluate the possibility (*5*).

As scientists with relevant expertise, we agree with the WHO director-general (*5*), the United States and 13 other countries (*6*), and the European Union (*7*) that greater clarity about the origins of this pandemic is necessary and feasible to achieve. We must take hypotheses about both natural and laboratory spillovers seriously until we have sufficient data. A proper investigation should be transparent, objective, data-driven, inclusive of broad expertise, subject to independent oversight, and responsibly managed to minimize the impact of conflicts of interest. Public health agencies and research laboratories alike need to open their records to the public. Investigators should document the veracity and provenance of data from which analyses are conducted and conclusions drawn, so that analyses are reproducible by independent experts.

Finally, in this time of unfortunate anti-Asian sentiment in some countries, we note that at the beginning of the pandemic, it was Chinese doctors, scientists, journalists, and citizens who shared with the world crucial information about the spread of the virus—often at great personal cost (*8*, *9*). We should show the same determination in promoting a dispassionate science-based discourse on this difficult but important issue.

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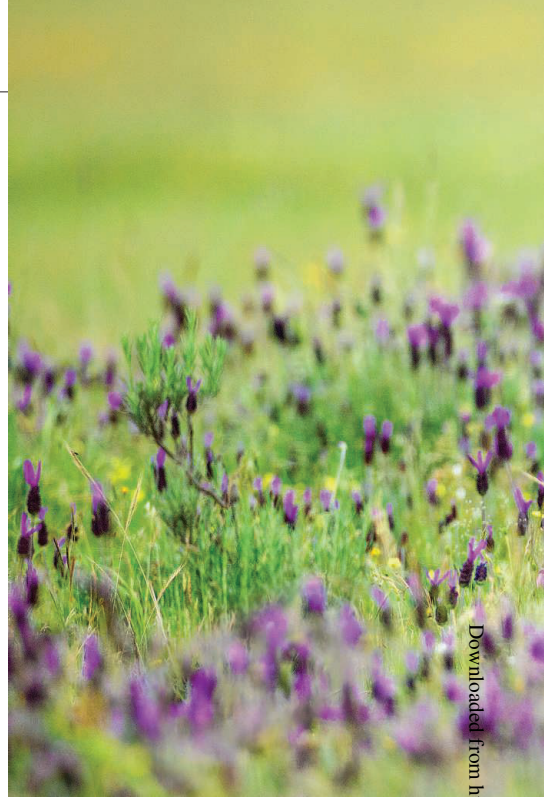

PHOTO: MARIO SUAREZ PORRAS/MINDEN PICTURES

Ban veterinary use of diclofenac in Europe

In Europe, vulture recovery has been an important conservation success story (*1*). This success may now be jeopardized by the use of diclofenac in Europe's pastoral landscapes. Although diclofenac had already caused a rapid and catastrophic 95% decline in Asian vulture populations (*2*), the non-steroidal anti-inflammatory drug was approved for veterinary use in Spain in 2013 (*3*). Although measures for the safe disposal of carcasses of livestock treated with diclofenac are supposed to prevent avian scavengers from feeding on contaminated carrion (*4*), a Spanish cinereous vulture (*Aegypius monachus*) was found dead,

A Spanish cinereous vulture (*Aegypius monachus*) was found poisoned by diclofenac in September 2020.

poisoned with diclofenac (*5*), in September 2020. European regulatory authorities should permanently ban diclofenac use in livestock before the tragedy met by Asian vultures repeats itself in Europe.

Vulture breeding populations in Spain represent more than 90% of the total European vulture population (*6*). Diclofenac use in livestock could contribute an additional annual mortality rate of 0.9% to 7.7% in Spanish griffon vultures (*7*). The vulture discovered in September was tracked by GPS tag. Given that untagged birds are harder to find, it is likely that more vultures have been poisoned by diclofenac but have not been found. The genus of the recently discovered bird is also ominous; previous diclofenac deaths have only affected species of the genus *Gyps* (*2*, *8*).

If bold measures are not immediately taken throughout Europe, the consequences for European vultures could be severe. In addition to posing an indirect threat, the legal availability of diclofenac may provide a highly efficient weapon to lawbreakers who wish vultures harm. European and national decision-makers should embrace a precautionary approach that promotes treating livestock with cost-effective, vulture-safe alternatives to diclofenac, such as meloxicam (*9*). These decisions would protect European avian scavengers and align with the new European Green Deal action plan for restoring biodiversity (*10*).

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Salmon aquaculture threatens Patagonia

In March, a massive die-off of farmed salmon sent more than 2.2 million kilos of rotting fish biomass into the fjords and channels of the Pacific Patagonian wilderness (*1*), critical areas for biodiversity conservation. The mass mortality event is part of a pattern in which industrial salmon farming increases eutrophication and boosts harmful micro-algae blooms (*2*), which enter gills and suffocate fish (*3*). In turn, decomposition of salmon carcasses leads to increased dissolved organic matter, which, in combination with human-induced ocean warming, facilitates the occurrence of more algal blooms (*4*). With a new constitutional act under discussion, Chile should seize this opportunity to add regulations that will stop the cycle and protect the valuable Patagonian region.

Pacific Patagonia remained mostly pristine until the 1980s (*5*). The region served as one of the last territories of thriving blue whales (*5*) and provided non-breeding habitat for long-distance migratory shorebirds breeding as far away as Alaska (*6*). Salmon aquaculture markedly changed this vast coastal landscape from Chiloé Archipelago to Tierra del Fuego, affecting even remote channels without any previous signs of human activity other than from Indigenous cultures (*7*). Despite repeated warnings regarding socio-environmental impacts (*8*), salmon aquaculture surpassed 1,000,000 tons in 2020 and is now one of the largest economic activities in Chile, the second-largest salmon producer in the world (*9*). In addition to pollution generated by the industry, the regular escape of farmed salmon from broken cages adds non-native mesopredators to food-webs and affects wildlife by transferring aquaculture-associated diseases (*10*) and antibiotic resistant bacteria and genes, which can take hold in wild animals (*11*).

The international community, which serves as the market for Chile's salmon, can leverage its economic power to convince Chile to take action to protect this unique biodiversity hotspot from the environmental effects of salmon aquaculture. Existing government regulations and industry standards must be strengthened. For example, current sustainable aquaculture labelling schemes label some salmon operations as "sustainable" without fully evaluating impacts to wildlife and the surrounding environment (*6*, *11*). The United Nations should push the Chilean government to halt the current expansion of salmon industry toward southern latitudes, especially in the Magallanes region, one of the last bastions of the Patagonian wilderness. Furthermore, a comprehensive monitoring program should be put in place to conduct annual reviews, give

warnings to the industry where necessary, and dismantle aquaculture operations that violate the regulations.

The United Nations should take advantage of the socio-political momentum in Chile. In October 2020, 79% of voters approved the creation of a new constitutional act for Chile, with the potential to address a variety of issues, including a wide range of environmental regulations (*12*). The proposed legislation presents an opportunity to place much-needed limits on aquaculture development. The act will take shape with the input of independent candidates rather than the current parliamentarians and senators who have contributed to the precarious aquaculture cycle. After three decades of salmon industry development, this process could finally lead to policies that protect the Pacific Patagonian wilderness.

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ERRATA

Erratum for the Report "Manta-like planktivorous sharks in Late Cretaceous oceans" by R. Vullo *et al.*, *Science* **372**, eabi9203 (2021). Published online 16 April 2021; 10.1126/science.abi9203

Investigate the origins of COVID-19

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Science, 372 (6543), • DOI: 10.1126/science.abj0016

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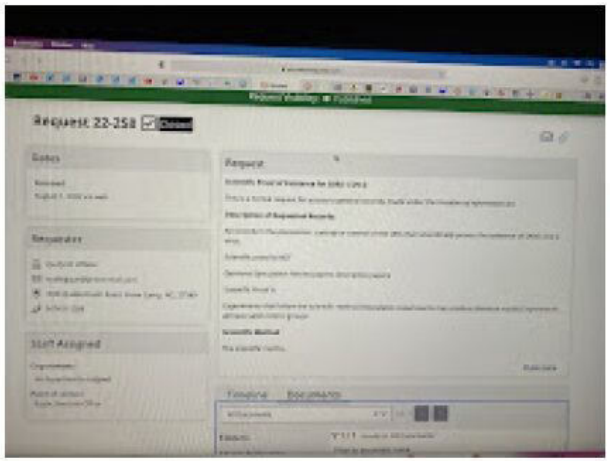

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This is a formal request for access to general records, made under the *Freedom of Information Act.*

Description of Requested Records:

All records in the possession, custody or control of the ORG that scientifically proves the existence of SARS-COV virus.

Scientific proof is NOT

Opinions Speculation Review papers Descriptive papers

Scientific Proof is

Experiments that follow the scientific method Repeatable experiments that produce identical results Experimer all have valid control groups

Scientific Method

The scientific metho...

Show m

Timeline Documents

All Documents 1-1 / 1

Folders

1 / 1 results in 'All Documents'

Filter by folder name

filter by folder name

Filter by document name

filter by document name

All Documents

Documents not in folders

Uploaded: 08/09/2022

pdf Req

2022.08.01 _ Wilson _ Response.pdf

Fwd: Re: Create a new password for the UNC-Chapel Hill public records portal

[redacted] Tue, Sep 13, 2022 at 1:17 PM
To: Christine Massey <cmssyc@gmail.com>

This is their response.

Sent from ProtonMail for iOS

---------- Forwarded message ----------
From: Public Records Office<publicrecordsoffice@email.unc.edu>
Date: On Tue, Sep 13, 2022 at 1:16 PM
Subject: Fwd: Re: Create a new password for the UNC-Chapel Hill public records portal
To: [redacted] Public Records Info <info@publicrecords.unc.edu>
Cc:

Thank you for your message. The University has provided all records responsive to this request.

Sincerely,

Public Records Office

From [redacted]
Date: Tuesday, September 13, 2022 at 11:19 AM
To: Public Records Info <info@publicrecords.unc.edu>
Subject: Re: Create a new password for the UNC-Chapel Hill public records portal

Some people who received this message don't often get email from healingque@protonmail.com. Learn why this is important

Thank you for your quick response.

The documents provided do not contain any scientific evidence of a physical particle found in humans. Nothing in them fits the description of a virus. Please go over the request again and if you have them provide the document showing evidence of its existence. If UNC does not have such records, please let me know.

Once again thank you so much for your time,

[redacted]

Sent with Proton Mail secure email.

------- Original Message -------
On Sunday, September 11th, 2022 at 10:10 AM, UNC-Chapel Hill Public Records Office <info@publicrecords.unc.edu> wrote:

You recently requested to reset your password for your UNC-Chapel Hill public records portal account.

Use the button below to reset it. **This password reset link is only valid for the next 24 hours.**

Reset Password

https://unc.nextrequest.com/users/password/edit?reset_password_token=[redacted]

If you did not request a password reset, please ignore this email. Your password won't change until you access the link above and create a new one.

NextRequest

an Optimere brand

The All in One Records Requests Platform

Technical support: See our help pageor email us at support@nextrequest.com

Too many emails? Change your email settings here