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Short communication

The dark side of COVID-19

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ABSTRACT

The origin of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is unknown. Studying it requires also identifying the origin of the most related coronaviruses. For this purpose, in full genomic analysis, we found three genomic fingerprints of the COVID-19 related coronavirus: at the beginning of the Orf1ab RNA replicase gene ; at S gene; and the NS8 gene itself, at the end of the genome. Only the Bat SARS-like coronavirus (Bat-SL-CoV, isolates ZXC21 and ZC45) genomes matched the COVID-19 genome extreme fingerprints. The S gene fingerprint was unmatched: it is the key to the SARS-CoV-2 origin. Further phylogenetic analysis showed an orthology relationship between BatCoV-RaTG13, SARS-CoV-2, Bat-SL-CoV and Panglolin-CoV (isolate MP789). This phylogenetic relationship was broken when the phylogenetic analysis was based on the spike glycoprotein sequences, with a high divergence of BatCoV-RaTG13 and SARS- CoV-2. However, the polybasic furin cleavage site in the SARS-CoV-2 spike glycoprotein made the difference from SARS-CoV-2 to BatCoV-RaTG13. Unfortunately, there is only one freely available genome of BatCoV-RaTG13 in Gen-Bank, both to statistically validate that 96% and to explore the remaining 4%. Because that polybasic furin cleavage site is responsible for the high SARS-CoV-2 infectivity and transmissibility, the availability of multiple BatCoV-RaTG13 complete genomes for comparative analysis would provide new perspective. Until this happens, the origin of SARS-CoV-2 is the dark side of COVID-19. KEY WORDS: COVID-19, SARS-CoV-2, BatCoV-RaTG13, Coronavirus, Bioinformatics

The origin of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus disease 2019 (COVID-19), is unknown. Its origin is linked to that of the most SARS-CoV-2 related coronaviruses. We addressed the issue through a bioinformatic approach, providing the following to previous knowledge.

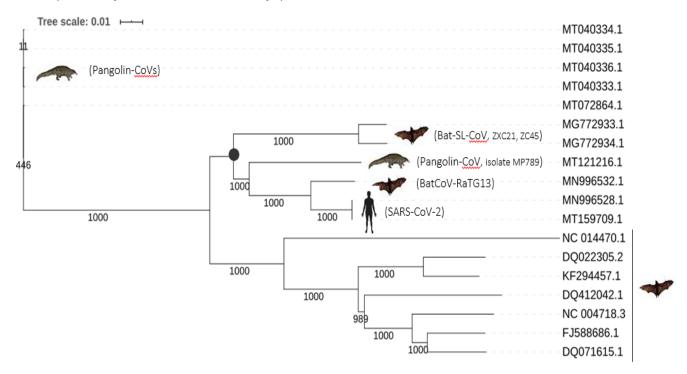
• First, using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI), there were clearly identified three genomic fingerprints of the COVID-19 related coronavirus: (i) the N-terminal Macro domain (ATP binding module) of the Orf1ab RNA replicase gene (based on SARS-CoV-2 genome, GenBank acession number MN996528.1, coordinates: 1940-3955); (ii) the N-terminal domain and receptor

binding domain (RBD) of the spike glycoprotein, S gene (genome coordinates, 21563-22963); and (iii) the NS8 gene itself (genome coordinates, 27912-28256). Only the sequences of the Bat SARS-like coronavirus (Bat-SL-CoV) (isolates ZXC21 and ZC45, GenBank accession numbers MG772934.1 and MG772933.1, respectively) gave a perfect match with the extremes genome fingerprints. On the other hand, the spike glycoprotein fingerprint was unmatched: it is the key to the SARS-CoV-2 origin.

• Second, the phylogenetic analysis based on complete genomes, showed that Bat- SL-CoV (ZXC21 and ZC45), Pangolin-CoV (isolate MP789, GenBank accession number MT121216.1), BatCoV-RaTG13 (GenBank accession number MN996532.1) and SARS-CoV-2 were orthologous. That is, they originated from a common ancestor coronavirus species, which were separated from each other after a speciation events. It is worth noting that not all pangolin coronaviruses are the same. Only the Panglolin-CoV (MP789) belonged to that group of orthologous coronavirus, other pangolin coronavirus were phylogenetically more distant (Figure 1).

Figure 1. Phylogenetic tree based on coronavirus complete genome

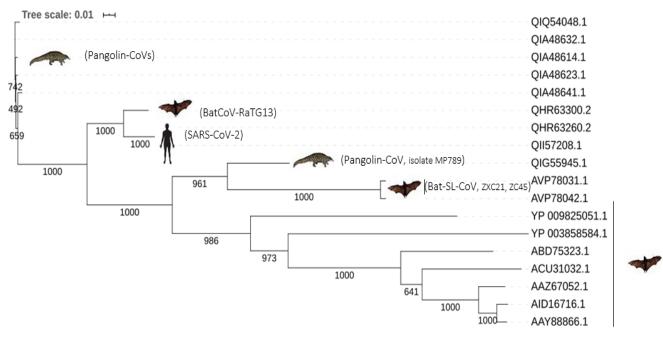
Phylogenetic tree based on a multialignment of complete genomes of selected coronaviruses. The sample includes the available pangolin coronaviruses, a selection of bat coronaviruses extracted from the literature (1,15,16), and two SARS-CoV-2 genomes as representatives of the NCBI "Severe acute respiratory syndrome coronavirus 2 (tax-id:2697049)" taxonomic group. The phylogenetic tree was constructed with the Neighbor Joining method of the Clustal Omega (v.1.2.4) software package using default parameters (17). Assessed clustering strength was calculated by bootstrap using 1000 replicates. Tree scale bar stands for the evolutionary distance, based on genome multialigment. The black point depicts the ancestral coronavirus species from which the group of homologous sequences were separated from each other after a speciation event. GenBank accession number of the complete genomes and the coronavirus were the following (in the same arrangement as in the phylogenetic tree): MT040333.1 to MT040336.1, Pangolin coronavirus; MG772933.1 and MG772934.1, Bat SARS-like coronavirus; MT121216.1 (isolate MP789) Pangolin coronavirus; MN996532.1, Bat coronavirus RaTG13; MN996528.1 and MT159709.1, SARS-CoV-2; NC_014470.1, Bat coronavirus BM48-31/BGR/2008; DQ022305.2, Bat SARS coronavirus HKU3-1; KF294457.1,Bat SARS-like coronavirus; DQ412042.1, Bat SARS CoV Rf1/2004; NC_004718.3, SARS coronavirus Tor2; FJ588686.1, SARS coronavirus Rs_672/2006; DQ071615.1, Bat SARS CoV Rp3/2004.



•Third, the phylogenetic relationship of orthologous was broken when the phylogenetic analysis was based on the spike glycoprotein sequences (specifically, from the N-terminal domain to the RBD, inclusive). In this new analysis, using the protein sequences from the same coronaviruses used in the analysis shown in Figure 1, the sequences of BatCoV-RaTG13 and SARS-CoV-2 diverged significantly from those of Bat-SL-CoV (ZXC21 and ZC45) and Pangolin-CoV (MP789). Furthermore, the last two were clearly grouped with the others bat coronaviruses (Figure 2). **Figure 2. Phylogenetic tree based on coronavirus spike glycoprotein**

Phylogenetic tree based on the multialignment of the most variable region of the coronavirus spike glycoprotein: from the N-terminal domain to the RBD, inclusive. In this phylogenetic analysis, we have tried to use the sequences of the same coronaviruses of Figure 1, with the intention of comparing the phylogenetic behavior, when it is based on the complete genome and when it is based on this strategic region of the genome. RBD position was based on (18). The phylogenetic tree was constructed with the Neighbor Joining method of the Clustal Omega (v.1.2.4) software package, using default parameters (17). Assesed clustering strength was calculated by bootstrap using 1000 replicates. Tree scale bar stands for the evolutionary distance, based on sequence multialignmet.GenBank accession number of the spike glycoprotein and the coronavirus were the following (in the same arrangement as in the phylogenetic tree): QIA48641.1, QIA48632.1, QIA48614.1, QIA48623.1, QIQ54048.1, Pangolin coronavirus; QHR63300.2, Bat coronavirus RaTG13; QHR63260.2, QII57208.1, SARS-CoV-2; QIG55945.1,

Pangolincoronavirus; AVP78031.1, AVP78042.1, Bat SARS-like coronavirus; YP_009825051.1, SARS coronavirus Tor2; YP_003858584.1, Bat coronavirus BM48-31/BGR/2008; ABD75323.1, Bat SARS CoV Rf1/2004; ACU31032.1, SARS coronavirus Rs_672/2006; AAZ67052.1, Bat SARS CoV Rp3/2004; AID16716.1, Bat SARS-like coronavirus; AAY88866.1, Bat SARS coronavirus HKU3-1.



BatCoV-RaTG13 has been considered likely to be the direct progenitor of SARS-CoV-2 (1), despite contact between humans and bats is limited (2). But, how and when did the Bat-CoV-RaTG13 appear? The BatCoV-RaTG13 genome was identified and sequenced by Zheng-Li Shi and coworkers, 2020 (1), within the framework of the study of a "new coronavirus", which caused the epidemic of acute respiratory syndrome in humans in Wuhan, China (December 2019). Literally: "We found that a short region of RNA- dependent RNA polymerase (RdRp) from a bat coronavirus (BatCoV RaTG13) —which was previously detected in Rhinolophus affinis from Yunnan province— showed high sequence identity to 2019-nCoV (SARS-CoV-2)". Then, Zheng-Li Shi and coworkers, 2020 (1), carried out full-length BatCoV-RaTG13 and SARS-CoV-2 genome sequencing, with an overall genome sequence identity of 96.2%. BatCoV-RaTG13 complete genome sequence was submitted to GenBank by Z.-L. Shi and coworkers on 27-JAN-2020 (MN996532.1). In the GenBank file, it appears as isolation source, fecal swab; host, Rhinolophus affinis; country, China; and collection date, 24-Jul-2013. From this available information, the origin of BatCoV-RaTG13 was prior to 2013. Accordingly, at least since 2013, it must be considered as an active bat coronavirus.

Regarding the spike glycoprotein phylogenetic divergence of BatCoV-RaTG13 and SARS-CoV-2, the RBD region of the spike glycoprotein is described as the most variable region of the coronavirus genomes (3). However, as it is shown in the multialignment (Figure 3), the N-terminal domain and RBD region is highly conserved, and many amino acid residues were strictly conserved. So that, according to Motoo Kimura's neutral theory of molecular evolution (4), in this critical region of the coronavirus genomes the balance between the random drift and the functional constraints would be enormously stressed. Nevertheless, the SARS-CoV-2 sipke glycoprotein appears to be optimized for binding to the human receptor ACE2 (3,5). Of course it has. Through The RBD of the spike glycoprotein attaches the virion to the cell membrane by interacting with host receptor, initiating the infection (5,6), and entry into human cells (7).

What evolutionary event could explain this RBD extraordinary optimization for human infection? A possible recombination event between BatCoV-RaTG13 and some bat coronavirus, or a horizontal gene transfer that had replaced variable regions in BatCoV- RaTG13 S gene, could be considered (8,9). But, either in recombination or a horizontal transfer, there always had to be a donor, and we could not identify any donor by analysing the entire NCBI nucleotide collection. This reasoning agrees with Zheng-Li Shi and coworkers, 2020 (1), literally: "Using the

aligned genome sequences of 2019-nCoV, RaTG13, SARS-CoV and previously reported bat SARSr-CoVs, no evidence for recombination events was detected in the genome of 2019-nCoV (SARS-CoV-2)". Alternatively, it could be assumed that at some point BatCoV-RatG13 S gene, had been subject to heavy selective pressure, however, the functional constraints exceed the random drift or random chance.

Figure 3. Coronavirus spike glycoprotein multiple sequence alignment.

Spike glycoprotein multiple sequence alignment. Groups of sequence sample: (i) human SARS coronavirus (SARS-CoV) (1255 amoniacids length); (ii) Bat-SL-CoV (1245); (iii) Pangolin-CoVs (1265-1269); (iv) SARS-CoV-2 (1273); and (v) BatCoV-RaTG13 (1269). To better visualize the characteristics of each group, there were three representative sequences of each. The protein alignments were created by Clustal Omega (v.1.2.4) using default parameters (18). Strictly conserved amino acids are denoted by *, gaps are denoted by -. The characteristic deletion and insertions of the COVID-19 related sequences, the RBD, and the SARS-CoV-2 polybasic furin cleavage site are highlighted in yellow. The color of the bands (gray-white) are intended to highlight the characteristics of: SARS-CoV; Bat-SL-CoV and Pangolin-CoV (MP789); other Pangolin-CoVs; SARS-CoV-2; and BatCoV-RaTG13. The RBD position was based on (19). The figure only show up to the furin cleavage site. Up to the C-terminal end, most positions were strictly conserved. GenBank accession number of the spike glycoprotein sequences and the coronavirus are the following: ADC35483.1, SARS coronavirus HKU-39849; sp|P59594| (UNIPROT SPIKE_CVHSA); AAR07630.1, SARS coronavirus BJ302; AVP78031.1, Bat-SL-CoV ZC45, MG772933.1; AVP78042.1, Bat-SL-CoV ZXC21, MG772934.1; QIG55945.1, Pangolin-CoV, MP789,MT121216.1; QIQ54048.1, Pang-CoV,GX-P2V,MT072864.1; QIA48614.1, Pang-CoV,GX-P4L,MT040333.1; QIA48623.1, Pang-CoV,GX- P1E,MT040334.1; QHR63260.2, SARS-CoV-2, MN996528.1; QII57208.1 SARS-CoV-2, MT04024.1

MT159709.2; QIA98554.1, SAR	S-CoV-2, MT066156.1; QHR63300.2, BatCoV-RaTG13, MN996532.1.	
ADC35483.1	-MFIFLLFLTLTSGSDLDRCTTFDDVOAPNYTOHTSSMRGVYYPDEIFRSDTLYLTODLF	59
sp P59594 SPIKE CVHSA	-MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLF	59
AAR07630.1	-MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLF	59
AVP78031.1	MLFFLFLQFALVNSOCVNLTGRTPLNPNYTNSSORGVYYPDTIYRSDTLVLSOGYF	56
AVP78042.1	MLFFLFLQFALVNSQC-DLTGRTPLNPNYTNSSQRGVYYPDTIYRSDTLVLSQGYF	55
QIG55945.1	MLFFFFLHFALVNSQCVNLTGRAAIQPSFTNSSQRGVYYPDTIFRSNTLVLSQGYF	56
QIQ54048.1	-MFVFLFVLPLVSSQCVNLTTRTGIPPGYTNSSTRGVYYPDKVFRSSILHLTQDLF	55
QIA48614.1	-MFVFLFVLPLVSSQCVNLTTRTGIPPGYTNSSTRGVYYPDKVFRSSILHLTQDLF	55
QIA48623.1	-MFVFLFVLPLVSSQCVNLTTRTGIQPGYTNSSTRGVYYPDKVFRSSILHLTQDLF	55
QHR63260.2	-MFVFLVLLPLVS3QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLF	55
QII57208.1	-MFVFLVLLPLVSS <mark></mark> QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLF	55
QIA98554.1	-MFVFLVLLPLVS3 <mark></mark> QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLF	55
QHR63300.2	-MFVFLVLLPLV33QCVNLTTRTQLPPAYTN3STRGVYYPDKVFR55VLHLTQDLF	55
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ADC35483.1	LPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQ	112
spiP59594 SPIKE_CVHSA	LPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQ	112
AAR07630.1	LPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQ	112
AVP78031.1	lpfysnvswyyslt <mark>tn-naat</mark> krtdnpildfkdgiyfaatehsniirgwifgttldnts <u>o</u>	115
AVP78042.1	lpfysnvswyyslt <mark>tn-naat</mark> krtdnpildfkdgiyfaatehsnivrgwifgttldnts <u>o</u>	114
QIG55945.1	LPFYSNVSWYYALT <mark>KT-NSAE</mark> KRVDNPVLDFKDGIYFAATEKSNIVRGWIFGTTLDNTSQ	115
QIQ54048.1	LPFFSNVTWFNTIH <mark>LNYQGGFKKFDNPVLPXNDGVYFASTEKSNIIRGWIFGTTLDARTQ</mark>	115
QIA48614.1	LPFFSNVTWFNTI- <mark>-NYQGGFKKFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDARTQ</mark>	113
QIA48623.1	LPFFSNVTWFNTINYQGGFKKFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDARTQ	113
QHR63260.2	lpffSnvtwfhaih <mark>v3gtngt</mark> krfdnpvlpfndgvyfastekSniirgwifgttldSktQ	115
QII57208.1	LPFFSNVTWFHAIH <mark>VSGTNGT</mark> KRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQ	115
QIA98554.1	LPFFSNVTWFHAIH <mark>VSGTNGT</mark> KRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQ	115
QHR63300.2	LPFFSNVTWFHAIH <mark>V3GTNGI</mark> KRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTO	115

ADC35483.1	SVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYISDAF	168
spiP5959413PIKE_CVH3A	SVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYISDAF	168
AAR07630.1	SVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYISDAF	168
AVP78031.1	SLLIVNNATNVIIKVCNFDFCYDPYLSGYYHN-N <mark>KTWS</mark> IREFAVYSSYANCTFEYVSKSF	174
AVP78042.1	SLLIVNNATNVIIKVCNFDFCYDPYLSGYYHN-N <mark>KTWS</mark> IREFAVYSFYANCTFEYVSKSF	173
QIG55945.1	SLLIVNNATNVIIKVCNFQFCYDPYLSGYYHN-N <mark>KTWS</mark> TREFAVYSSYANCTFEYVSKSF	174
QIQ54048.1	SLLIVNNATNVVIKVCEFQFCTDPFLGVYYHNNNKTWVENEFRVYSSANNCTFEYISQPF	175
QIA48614.1	SLLIVNNATNVVIKVCEFQFCTDPFLGVYYHNNNKTWVENEFRVYSSANNCTFEYISQPF	173
QIA48623.1	SLLIVNNATNVVIKVCEFQFCTDPFLGVYYHNNNKTWVENEFRVYSSANNCTFEYISQPF	173
QHR63260.2 QII57208.1	SLLIVNNATNVVIKVCEFOFCNDPFLGVYYHKNN <mark>KSWM</mark> ESEFRVYSSANNCTFEYVSOPF SLLIVNNATNVVIKVCEFOFCNDPFLGVYYHKNN <mark>KSWM</mark> ESEFRVYSSANNCTFEYVSOPF	175 175
Q1157208.1 OIA98554.1	SLLIVNNATNVVIKVCEPOFCNDPFLGVYYHKNN <mark>KSWM</mark> ESEFRVYSSANNCTFEYVSOPF SLLIVNNATNVVIKVCEFOFCNDPFLGVYYHKNNK <mark>KSWM</mark> ESEFRVYSSANNCTFEYVSOPF	175
QIA98554.1 OHR63300.2	SLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNN KSWMESEFRVYSSANNCTFEYVSOPF SLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNN KSWMESEFRVYSSANNCTFEYVSOPF	175
Anu good a	SLLIVAARIAVVIAVCEPUPCADFFLGVIIRKAA KSWAESEFRVISSRANCIFEIVSOFF	1/5

ADC35483.1	SLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYOPIDVVRDLPSGFNTLKPIFKLPLGINI	228
sp P59594 SPIKE_CVHSA	SLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYOPIDVVRDLPSGFNTLKPIFKLPLGINI	228
AAR07630.1	SLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINI	228
AVP78031.1	MLNISGNGGLFNTLREFVFRNVDGHFKIYSKFTPVNLNRGLFTGLSVLQPLVELFVSINI	234
AVP78042.1	MLNISGNGGLFNTLREFVFRNVDGHFKIYSKFTPVNLNRGLPTGLSVLQPLVELPVSINI	233
QIG55945.1	MLDIAGKSGLFDTLREFVFRNVDGYFKIYSKYTPVNVNSNLPIGFSALEPLVEIPAGINI	234
QIQ54048.1	LMDLEGKQGNFKNLREFVFKNVDGYFKIYSKHTPIDLVRDLPRGFAALEPLVDLPIGINI	235
QIA48614.1	LMDLEGKQGNFKNLREFVFKNVDGYFKIYSKHTPIDLVRDLPRGFAALEPLVDLPIGINI	233
QIA48623.1	LMDLEGKQGNFKNLREFVFKNVDGYFKIYSKHTPIDLVRDLPRGFAALEPLVDLPIGINI	233
QHR63260.2	LMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINI	235
QII57208.1	LMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINI	235
QIA98554.1	LMDLEGKOGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPOGFSALEPLVDLPIGINI	235
QHR63300.2	LMDLEGKOGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPPGFSALEPLVDLPIGINI	235
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ADC35483.1	TNFRAILTAFSPAODIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSONP	282
spiP595941SPIKE_CVHSA	TNFRAILTAFSPAODIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSONP	282
AAR07630.1	TNFRAILTAFS PAODTWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSONP	282
AVP78031.1	TKFRTLLTIHRGDPMPNNGWTAFSAAYFVGYLKPRTFMLKYNENGTITDAVDCALDP	291
AVP78042.1	TKFRTLLTIHRGDFM3NNGWTAFSAAYFVGYLKPRTFMLKYNENGTITDAVDCALDP	290
01G55945.1	TKFRTLLTIHRGDFMFNNGWTVFSAAYYVGYLAPRTFMLNYNENGTITDAVDCALDP	291
QIQ54048.1	TRFOTLLALHRSYLTPGKLESGWTTGAAAYYVGYLOORTFLLSYNONGTITDAVDCSLDP	295
QIA48614.1	TRFOTLLALHRSYLTFGNLESGWTTGAAAYYVGYLOORTFLLSYNONGTITDAVDCSLDP	293
QIA48623.1	TRFQTLLALHRSYLTPGKLESGWTTGAAAYYVGYLQQRTFLLSYNQNGTITDAVDCSLDP	293
OHR63260.2	TRFOTLLALHRSYLTFGD355GWTAGAAAYYVGYLOPRTFLLKYNENGTITDAVDCALDP	295
01157208.1	TRFOTLLALHRSYLTFGD355GWTAGAAAYYVGYLOPRTFLLKYNENGTITDAVDCALDP	295
0IA98554.1	TRFOTLLALHRSYLTPGD355GWTAGAAAYYVGYLOPRTFLLKYNENGTITDAVDCALDP	295
OHR63300.2	TRFQTLLALHRSYLTPGD355GwTAGAAAYYVGYLOPRTFLLKYNENGTITDAVDCALDF	295
2	1.1:::1:	
ADC35483.1	LAELKC3VK3FEIDKGIYQT3NFRVVP3GDVVRFPNITNLCPFGEVFNATKFP3VYAWER	342
spiP5959413PIKE_CVHSA	LAELKCSVKSFEIDKGIYOTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWER	342
AAR07630.1	LAELKCSVKSFEIDKGIYOTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVIAWER	342
AVP78031.1	LSETKCTLKSLTVQKGIYQTSNFRVQPTQSVVRFPNITNVCPFHKVFNATRFPSVYAWER	351
AVP78042.1	LSETKCILKSLIVQKGIIQISNIKVQPIQSVVRPPNIINVCPPHKVPNATRPSVIAWER	351
OIG55945.1	LSEAKCTLKSLSVQKGI IQISNFRVQPIQSI <mark>VRPMIINVCPINKVFRAIRFPSVIANER</mark> LSEAKCTLKSLTVEKGI YQTSNFRVQPIESI <mark>VRPPNIINLCPFGEVFNAIFFPSVIANNR</mark>	350
QIQ54048.1	LSETKCTLKSLTVEKGIYQTSNFRVQFTESIVRFPNITNLCPFGEVFNASKFASVYAWNR	355
QIQ34048.1 OIA48614.1	LSETKCILKSLIVEKGIYOTSNFRVOFTISIVRFPNITNLCFFGEVFNASKFASVYAWNR	353
QIA48623.1	LSETKCILKSLIVEKGIYOTSNFRVQFIISIVRFPNITNLCFFGEVFNASKFASVYAWNR	353
OHR63260.2	LSETKCTLKSFTVEKGIYOTSNFRVOPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR	355
QII57208.1	LSETKCILKSFIVEKGIYOTSNFRVOPTESIVRFPNITNLCPFGEVFNATRFASVIAWAR	355
Q1157208.1 QIA98554.1	LSETKCILKSFIVEKGIYQTSNFRVQFIESIVRFPNIINLCPFGEVFNATRFASVIAWNR LSETKCILKSFIVEKGIYQTSNFRVQFTESIVRFPNIINLCPFGEVFNATRFASVYAWNR	355
OHR63300.2	LSETKCTLKSFTVEKGIYOTSNFRVOPTESTVRFPNTTNLCPFGEVFNATTFASVYAWNR	355
Quinesses.5		300
10000000		
ADC35483.1	KKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQT	402
sp P59594 SPIKE_CVHSA	KKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQT	402
AAR07630.1	KKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVROIAPGOT	402
AVF78031.1	TKISDCIADYTVFYNSTSFSTFKCYGVSPSKLIDLCFTSVYADTFLIRFSEVROVAPGOT	411
AVP78042.1		
	TKISDCIADYTVFYNSTSFSTFKCYGVSPSKLIDLCFTSVYADTFLIRFSEVRQVAPGQT	410
QIG55945.1	KRISNCVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVVRGDEVRQIAPGQT	411
QIQ54048.1	KRISNCVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVVKGDEVRQIAPGQT	415
QIA48614.1	KRISNCVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVVKGDEVRQIAPGQT	413
QIA48623.1	KRISNCVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVVKGDEVROIAPGOT	413
-		
QHR63260.2	KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQT	415
QII57208.1	KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQT	415
QIA98554.1	KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQT	415
OHR63300.2	KRISNCVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVITGDEVROIAPGOT	415

ADC35483.1	GVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSP	462
sp P59594 SPIKE_CVHSA	GVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSP	462
AAR07630.1	GVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSP	462
AVP78031.1	GVIADYNYKLPDDFTGCVIAWNTAKQDVGNYFYRSHRSTKLKPFERDLSSDEN	464
AVP78042.1	GVIADYNYKLPDDFTGCVIAWNTAKQDTGHYFYRSHRSTKLKPFERDLSSDEN	463
QIG55945.1	GRIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQA	471
QIQ54048.1	GVIADYNYKLPDDFTGCVIAWNSVKODALTGGNYGYLYRLFRKSKLKPFERDISTEIYOA	475
QIA48614.1	GVIADYNYKLPDDFTGCVIAWNSVKODALTGGNYGYLYRLFRKSKLKPFERDISTEIYOA	473
OIA48623.1	GVIADYNYKLPDDFTGCVIAWNSVKODALTGGNYLYRLFRKSKLKPFERDISTEIYOA	471
OHR63260.2	GKIADYNYKLPDDFIGCVIAWNSVRODALIGGAI - LIKLFRKSRLKFFERDISILIIGA GKIADYNYKLPDDFIGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKFFERDISILIYOA	475
OII57208.1		
	GKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQA	475
QIA98554.1	GKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQA	475
QHR63300.2	GKIADYNYKLPDDFTGCVIAWNSKHIDAKEGGNFNYLYRLFRKANLKPFERDISTEIYQA	475
ADC35483.1	DGKPCT-PPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIK	521
sp P59594 SPIKE_CVHSA	DGKPCT-PPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIK	521
AAR07630.1	DGKPCT-PPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIK	521
AVP78031.1	GVRTLSTYDFNPNVPLEYOATRVVVLSFELLNAPATVCGPKLSTOLVK	512
AVP78042.1	GVRTLSTYDFNPNVPLEYOATRVVVLSFELLNAPATVCGPKLSTOLVK	511
0IG55945.1	GST PCNGVEGFNCYFPLOSYGFHPTNGVGYOPYRVVVLSFELLKAPATVCGPKOSTNLVK	531
01054048.1	GSTPCNGOVGINCYPLERYGFHPTTGVNYOPFRVVVLSXELLNGPATVCGPKLSTTLVK	
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QIA48614.1	GSTPCNGQVGLNCYYPLERYGFHPTTGVNYQPFRVVVLSFELLNGPATVCGPKLSTTLVK	533
QIA48623.1	GSTPCNGQVGLNCYYPLERYGFHPTTGVNYQPFRVVVLSFELLNGPATVCGPKLSTTLVK	531
QHR63260.2	GSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVK	535
QII57208.1	GSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVK	535
QIA98554.1	GSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVK	535
OHR63300.2	GSKPCNGQTGLNCYYPLYRYGFYPTDGVGHQPYRVVVLSFELLNAPATVCGPKKSTNLVK	535
ADC35483.1	NOCVNFNFNGLTGTGVLTP33KRF0PF00FGRDV3DFTD3VRDPKT3EILDI3PC3FGGV	581
sp P59594 SPIKE CVHSA	NQCVNFNFNGLTGTGVLTPSSKRFQFFQQFGRDVSDFTDSVRDFKTSEILDISPCSFGGV	581
AAR07630.1	NOCVNFNFNGLTGTGVLTPSSKRFOPFOOFGRDVSDFTDSVRDPKTSEILDISPCSFGGV	581
AVP78031.1	NQCVNFNFNGLKGTGVLTDSSKRFQSFQQFGKDASDFIDSVRDPQTLEILDITPCSFGGV	572
AVP78042.1	NQCVNFNFNGLKGTGVLTDSSKRFQSFQQFGKDASDFIDSVRDPQTLEILDITPCSFGGV	571
QIG55945.1	NKCVNFNFNGLTGTGVLTESSKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGV	591
QIQ54048.1	DKCVNFNFNGLTGTGVLTTSKKQFLPFQQFGRDISDTTDAVRDPQTLEILDITPC3FGGV	595
QIA48614.1	DKCVNFNFNGLTGTGVLTTSKKOFLPFOOFGRDISDTTDAVRDPOTLEILDITPCSFGGV	593
OIA48623.1	DKCVNFNFNGLTGTGVLTTSKKOFLPFOOFGRDISDTTDAVRDPOTLEILDITPCSFGGV	591
OHR63260.2	NKCVNFNFNGLTGTGVLTESNKKFLPFOOFGRDIADTTDAVRDFOTLEILDITPCSFGGV	595
01157208.1	NKCVNFNFNGLTGTGVLTESNKKFLPFQQFGKDIADTTDAVRDPQTLEILDITPCSFGGV	595
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QIA98554.1	NKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGV	595
QHR63300.2	NKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGV	595
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ADC35483.1	SVITPGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEH	641
sp P59594 SPIKE_CVHSA	SVITPGTNASSEVAVLYODVNCTDVSTAIHADOLTPAWRIYSTGNNVFOTOAGCLIGAEH	641
AAR07630.1	SVITPGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEH	641
AVP78031.1 AVP78042.1	SVITPGTNTSLEVAVLYQDVNCTDVPTTIHADQLTPAWRIYATGTNVFQTQAGCLIGAEH SVITPGTNTSSEVAVLYQDVNCTDVPTTIHADQLTPAWRIYAIGTSVFQTQAGCLIGAEH	632
QIG55945.1	SVITPGINISSEVAULIODVNCTEVPVAIHADOLTPTWRVYSTGSNVFOTRAGCLIGAEH	631 651
QIQ54048.1	SVITPGINISNQVAVLYQDVNCTEVPMAIHAEQLTPAWRVYSAGANVFQTRAGCLVGAEH	655
QIA48614.1	SVITPGTNTSNOVAVLYODVNCTEVPMAIHAEOLTPAWRVYSAGANVFOTRAGCLVGAEH	653
QIA48623.1	SVITPGTNTSNOVAVLYODVNCTEVPMAIHAEOLTPAWRVYSAGANVFOTRAGCLVGAEH	651
QHR63260.2	SVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEH	655
QII57208.1	SVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEH	655
QIA98554.1	SVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEH	655
QHR63300.2	SVITPGTNASNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEH	655
ADC35483.1	VDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIP	697
spiP5959413PIKE_CVHSA	VDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIP	697
AAR07630.1	VDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIP	697
AVP78031.1	VNASYECDIPIGAGICASYHTASI <mark>L</mark> RSTSQKAIVAYTMSLGAENSIAYANNSIAIP	688
AVP78042.1	VNASYECDIPIGAGICASYHTASI <mark>I</mark> RSTGQKAIVAYTMSLGAENSIAYANNSIAIP	687
QIG55945.1	VNNTYECDIPIGAGICASYQTQTNSRSVSSQAIIAYTMSLGAENSVAYANNSIAIP	707
QIQ54048.1	VNNSYECDIPVGAGICASYHSMSSFRSVNQRSIIAYTMSLGAENSVAYSNNSIAIP VNNSYECDIPVGAGICASYHSMSSLRSVNQRSIIAYTMSLGAENSVAYSNNSIAIP	711 709
QIA48614.1 QIA48623.1	VNNSYECDIPVGAGICASYHSMSSLRSVNQRSIIAYIMSLGAENSVAYSNNSIAIP VNNSYECDIPVGAGICASYHSMSSLRSVNQRSIIAYIMSLGAENSVAYSNNSIAIP	709
OHR63260.2	VNNSHECHIFVGAGICASYOTOTNSPRRARSVASOSIIAYTMSLGAENSVAYSNNSIAIP	715
QII57208.1	VNNSYECDIPIGAGICASYOTOTN <mark>SPRRA</mark> RSVASOSIIAYTMSLGAENSVAYSNNSIAIP	715
QIA98554.1	VNNSYECDIPIGAGICASYOTOTN <mark>SPRRA</mark> RSVASOSIIAYTMSLGAENSVAYSNNSIAIP	715
QHR63300.2	VNNSYECDIPIGAGICASYQTQTN <mark>S</mark> RSVASQSIIAYTMSLGAENSVAYSNNSIAIP	711

Using a forensic genetic criterion, which is valid in a jury, in this case of the SARS-CoV-2 (due the presence of the polybasic furin cleavage site), there is a likelihood ratio (LR) that is "infinite". That is, the relationship between the probability (P1) that given the SARS-CoV- 2 genome sequence, this sequence belongs to SARS-CoV-2, obviously, it is 1; and the probability (P2) that given the same SARS-CoV-2 genome sequence, it belongs to another coronavirus of its taxonomic group, at the moment it is 0 (because the polybasic furin site has only been found in SARS-CoV-2). Then that LR is infinite (LR = P1/P2 = 1/0 = infinite). It is a probabilistic way that shows how difficult is to associate SARS-CoV-2 with lineage B of the beta-coronaviruses. In this sense, Z.-L. Shi and coworkers, 2020 (1), (not taking into account the furin site), also pointed out this doubt, literally: "Phylogenetic analysis of the full-length genome and the gene sequences of RdRp and spike (S) showed that—for all sequences—RaTG13 is the closest relative of 2019-nCoV (SARS-CoV-2) and they form a distinct lineage from other SARS-CoVs".

Turning now to the the origin of the SARS-CoV-2, it must be taken into account that in the world of viruses the basic principles of biology are also fulfilled. From the Cell Theory of Rudolf Virchow (1858), "Omnis cellula ex cellula" (each cell derived from another pre- existing cell), it could be inferred as "each virus derives from a pre-existing virus". The Natural Selection of Charles Darwin (1859) of change mutations in the struggle for existence is fully applicable to the viruses. The principle of Theodosius Dobzhansky (1973) "Nothing in Biology Makes Sense Except in the Light of Evolution" (13) must be also applicable to viruses.

In science, and in biology, there have always been, there are and will be doubts and enigmas to be solved. Then, it is surprising that after almost a year of the pandemic, no more coronaviruses of BatCoV-RaTG13 species have been isolated. The bat (the natural virus reservoir most studied) strains could be isolated from fecal swab, and their complete genomes could be submitted to Gen-Bank database for analysis by the scientific community. At least, this would serve to validate statistically the 96% genome identity with SARS-CoV-2. There is still 4%, which may hold the key to the presence of the polybasic furin site in the SARS-CoV-2 spike glycoprotein. As for SARS-CoV-2, there are thousands of complete genomes available in the database.

In biological evolution there are always doubts and "missing links", that based on the discovery of new fossil remains, could be cleared or resolved. However, doubt about the origin of SARS-CoV-2 is serious, because the doubt may include the possibility that its origin could be a laboratory construct or a purposefully manipulated virus. The right technology for this is available. Thus, for scientific and medical purposes, several synthetic constructs of SARS-CoV-2 genome exist in GenBank database (accession number MT108784.1, MT461669.1, MT461671.1, MT461670.1), or for therapeutic purposes, synthetic recombinant bat SARS-like coronavirus was created, and was infectious in cultured cells and in mice (14).

Finally, the availability of multiple BatCoV-RaTG13 complete genomes for comparative analysis would provide new perspective. Until this happens, the origin of SARS-CoV-2 is the dark side of COVID-19. There is reasonable hope, just for probability and because the natural "trial-error" evolutionary mechanism, that a human virus like SARS-CoV-2 will not appear again for a long, long time (nobody gets the lottery jackpot twice). Now we have to wait for an upcoming vaccine and/or specific therapeutic drugs available for COVID-19.

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A.R. and E.O. performed project planning, coordination, execution, and facilitation. A.R. processed data collection and sequence and phylogenetic analysis. A.R. and E.O. prepared the manuscript. **Competing interest declaration**

All authors declare that they have no conflicts of interest.

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