Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

International Journal of Biological Macromolecules 191 (2021) 934–955

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Implications derived from S-protein variants of SARS-CoV-2 from six continents

Sk. Sarif Hassan [a,*], Kenneth Lundstrom [b], Debmalya Barh [c,d], Raner Jośe Santana Silva [e], Bruno Silva Andrade [f], Vasco Azevedo [g], Pabitra Pal Choudhury [h], Giorgio Palu [i], Bruce D. Uhal [j], Ramesh Kandimalla [k,l], Murat Seyran [m], Amos Lal [n], Samendra P. Sherchan [o], Gajendra Kumar Azad [p], Alaa A.A. Aljabali [q], Adam M. Brufsky [r], Ángel Serrano-Aroca [s], Parise Adadi [t], Tarek Mohamed Abd El-Aziz [u,v], Elrashdy M. Redwan [w,x], Kazuo Takayama [y], Nima Rezaei [z,aa], Murtaza Tambuwala [ab], Vladimir N. Uversky [ac,ad,*]

[a] Department of Mathematics, Pingla Thana Mahavidyalaya, Maligram, Paschim Medinipur 721140, West Bengal, India
[b] PanTherapeutics, Rte de Lavaux 49, CH1095 Lutry, Switzerland
[c] Centre for Genomics and Applied Gene Technology, Institute of Integrative Omics and Applied Biotechnology (IIOAB), Nonakuri, Purba Medinipur, WB, India
[d] Department of Genetics, Ecology and Evolution, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte 31270-901, Brazil
[e] Department of Biological Sciences (DCB), Graduate Program in Genetics and Molecular Biology (PPGGBM), State University of Santa Cruz (UESC), Rodovia Ilheus-Itabuna, km 16, 45662-900 Ilheus, BA, Brazil
[f] Laboratory of Bioinformatics and Computational Chemistry, Department of Biological Sciences, State University of Southwest Bahia (UESB), Jequié 45206-190, Brazil
[g] Laborat'orio de Geńetica Celular e Molecular, Departamento de Genetica, Ecologia e Evolucao, Instituto de Ciencias Biol'ogicas, Universidade Federal de Minas Gerais, Belo Horizonte CEP 31270-901, Brazil
[h] Applied Statistics Unit, Indian Statistical Institute, 203 B T Road, Kolkata 700108, India
[i] Department of Molecular Medicine, University of Padova, Via Gabelli 63, 35121 Padova, Italy
[j] Department of Physiology, Michigan State University, East Lansing, MI 48824, USA
[k] Applied Biology, CSIR-Indian Institute of Chemical Technology, Uppal Road, Tarnaka, Hyderabad 500007, India
[l] Department of Biochemistry, Kakatiya Medical College, Warangal, Telangana, India
[m] Doctoral Studies in Natural and Technical Sciences (SPL 44), University of Vienna, Wahringer Straße, A-1090 Vienna, Austria
[n] Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN, USA
[o] Department of Environmental Health Sciences, Tulane University, New Orleans, LA 70112, USA
[p] Department of Zoology, Patna University, Patna, Bihar, India
[q] Department of Pharmaceutics and Pharmaceutical Technology, Yarmouk University, Faculty of Pharmacy, Irbid 566, Jordan
[r] University of Pittsburgh School of Medicine, Department of Medicine, Division of Hematology/Oncology, UPMC Hillman Cancer Center, Pittsburgh, PA, USA
[s] Biomaterials and Bioengineering Lab, Centro de Investigaci'on Traslacional San Alberto Magno, Universidad Católica de Valencia San Vicente Ḿartir, c/Guillem de Castro, 94, 46001 Valencia, Spain
[t] Department of Food Science, University of Otago, Dunedin 9054, New Zealand
[u] Zoology Department, Faculty of Science, Minia University, El-Minia 61519, Egypt
[v] Department of Cellular and Integrative Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229-3900, USA
[w] Faculty of Science, Department of Biological Science, King Abdulazizi University, Jeddah 21589, Saudi Arabia
[x] Therapeutic and Protective Proteins Laboratory, Protein Research Department, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technology Applications, New Borg El-Arab, Alexandria 21934, Egypt
[y] Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto 606-8507, Japan
[z] Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
[aa] Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Stockholm, Sweden
[ab] School of Pharmacy and Pharmaceutical Science, Ulster University, Coleraine BT52 1SA, Northern Ireland, UK
[ac] Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL 33612, USA
[ad] Center for Molecular Mechanisms of Aging and Age-Related Diseases, Moscow Institute of Physics and Technology, Institutskiy pereulok, 9, Dolgoprudny, 141700, Russia

* Corresponding authors.
E-mail addresses: sarimif@gmail.com (Sk.S. Hassan), dr.barh@gmail.com (D. Barh), bandrade@uesb.edu.br (B.S. Andrade), vascoariston@gmail.com (V. Azevedo), giorgio.palu@unipd.it (G. Palu), sshercha@tulane.edu (S.P. Sherchan), gkazad@patnauniversity.ac.in (G.K. Azad), alaaj@yu.edu.jo (A.A.A. Aljabali), brufskyam@upmc.edu (A.M. Brufsky), angel.serrano@ucv.es (Á. Serrano-Aroca), mohamedt1@uthscsa.edu (T.M. Abd El-Aziz), lradwan@kau.edu.sa (E.M. Redwan), kazuo.takayama@cira.kyoto-u.ac.jp (K. Takayama), rezaei_nima@tums.ac.ir (N. Rezaei), m.tambuwala@ulster.ac.uk (M. Tambuwala), vuversky@usf.edu (V.N. Uversky).

https://doi.org/10.1016/j.ijbiomac.2021.09.080
Received 15 August 2021; Received in revised form 13 September 2021; Accepted 13 September 2021
Available online 24 September 2021

ARTICLE INFO

Keywords:
SARS-CoV-2
Invariant residues
Mutations
Spike protein
Continents
Vaccines

ABSTRACT

The spike (S) protein is a critical determinant of the infectivity and antigenicity of SARS-CoV-2. Several mutations in the S protein of SARS-CoV-2 have already been detected, and their effect in immune system evasion and enhanced transmission as a cause of increased morbidity and mortality are being investigated. From pathogenic and epidemiological perspectives, S proteins are of prime interest to researchers. This study focused on the unique variants of S proteins from six continents: Asia, Africa, Europe, Oceania, South America, and North America. In comparison to the other five continents, Africa had the highest percentage of unique S proteins (29.1%). The phylogenetic relationship implies that unique S proteins from North America are significantly different from those of the other five continents. They are most likely to spread to the other geographic locations through international travel or naturally by emerging mutations. It is suggested that restriction of international travel should be considered, and massive vaccination as an utmost measure to combat the spread of the COVID-19 pandemic. It is also further suggested that the efficacy of existing vaccines and future vaccine development must be reviewed with careful scrutiny, and if needed, further re-engineered based on requirements dictated by new emerging S protein variants.

1. Introduction

The world is experiencing a health emergency due to the Coronavirus disease (COVID-19), caused by an enveloped positive-sense single-stranded virus, the severe acute respiratory syndrome coronavirus (SARS-CoV-2) [1–6]. The spike (S) protein is a homotrimer present on the surface of the SARS-CoV-2 and recognizes the human host cell surface receptor angiotensin-converting enzyme-2 (ACE2) [7–10]. The interaction between the S protein of SARS-CoV-2 and its cellular receptor ACE2 is driven by high affinity/avidity. Therefore, neutralization by antibodies does not only require specifically binding antibodies, but antibodies that have high affinity/avidity towards the S1 subunit of the S protein [11]. It is worth mentioning that this particular aspect is directly related to the variability of the S1 subunit (and its isoelectric points) as this may modulate the affinity of binding [12]. The importance of antibody avidity for protection towards SARS-CoV-2 (and other viruses) has been recently reviewed [12]. From the beginning of the second wave of COVID-19 infection, various SARS-CoV-2 variants emerged raising concern of enhanced transmission and mortality of the virus and reduced efficacy of vaccine protection [13,14]. Some of the studies opposed the perception of SARS-CoV-2 mutations as distinctive pathogenic variants and the increased rate of transmissibility were questioned [15,16]. However, the frequency of the mutant strains within the SARS-CoV-2 population carrying the D614G mutation in the S protein clearly plays a role in enabling the virus to spread more effectively and rapidly [17]. Epidemiologists have been constantly monitoring the evolution of SARS-CoV-2 with a particular focus on the S protein and other interacting proteins of the virus [17,18]. The D614G mutation in the S protein discovered in early 2020 makes the virus able to spread more effectively and rapidly [19]. The D614G mutation has been found to be related with high viral loads in infected patients, and high rate of infections, but not with increased disease severity [20]. Various mutations in the S protein make the SARS-CoV-2 more complex and hence it is more difficult to characterize its severity, infectivity and efficacy of vaccines designed to target the S protein. Not all mutations are advantageous to the virus but several mutations or a set of mutations may increase the transmission potential through an increase in receptor binding or the ability to evade the host immune response by altering the surface structures recognized by antibodies [21–23].

To contain the spread of COVID-19, it is definitely of high interest to detect and identify various unique emerging variants of S proteins. Additionally, it is also worth investigating the impact of new S protein variants on viral infectivity and potential to spread rapidly as well as to ascertain the origin of the spread of the new variants concerning S protein variabilities. Accordingly, it might be possible to segregate the set of new variants with respect to individual characteristics of SARS-CoV-2, which would undoubtedly help policy makers to form various strategies to contain the spread of the virus. There are a large number of different SARS-CoV-2 S protein mutant sequences currently available in the National Center for Biotechnology Information (NCBI) virus database. In this study, all available S protein sequences from six continents Asia, Africa, Europe, North America, South America, and Oceania were analyzed for their uniqueness and variability. An inter-linkage was made among the unique S proteins available on the six continents.

2. Data acquisition and methods

S protein sequences from all six continents (Asia, Africa, Europe, Oceania, South America, and North America) were downloaded in FASTA format from the NCBI database (http://www.ncbi.nlm.nih.gov/). Further, FASTA files were processed in Matlab-2021a for extracting unique S protein sequences for each continent.

2.1. Phylogenetic analysis

To filter sequences with low quality (unknown amino acids 'X') and remove redundant sequences, the SeqKit tool was used, with the tools fx2tab and rmdup, respectively [24]. The filter removed all sequences that had one or more 'X' and all redundant sequences (100% identical). The amino acid sequences were aligned using the MegaX program with MUSCLE algorithm, and after it a phylogeny calculation was performed with the Neighbor-joining method, considering 3919 taxa sequences and 530 sites [25,26]. The alignment was used as input in Archeopteryx 0.9914 with the multiple alignment inference option, following the parameters of maximum allowed gaps ratio 0.5, minimum allowed non-gap sequence length 50 and distance calculator Kimura correction [27]. The phylogenetic trees were analyzed and edited in the Archeopteryx 0.9914 tool.

2.2. Frequency probability of amino acids

Any protein sequence is composed of twenty different amino acids with various frequencies starting from zero. The ability of occurrence of each amino acid Ai is determined by the formula $\frac{f(A_i)}{l}$ where $f(A_i)$ denotes the frequency of occurrence of the amino acid A_i in a primary sequence, and l stands for the length of an S protein [28]. Hence for each S protein,

a twenty-dimensional vector considering the frequency probability of twenty amino acids can be obtained. Based on this frequency probability, the dominance of amino acid density in a given protein is illuminated.

2.3. Evaluation of normalized amino acid compositions

The variability of the amino acid compositions of the unique S proteins from each continent was evaluated using the web-based tool Composition Profiler (http://www.cprofiler.org/) that automates detection of enrichment or depletion patterns of individual amino acids or groups of amino acids in query proteins [29]. In this analysis, we used sets of unique S proteins from each continent as query samples and the amino acid of the original S protein (UniProt ID: P0DTC2) as a reference sample that provides the background amino acid distribution. Composition profiler generates a bar chart composed of twenty data points (one for each amino acid), where bar heights indicate normalized enrichment or depletion of a given residue. The normalized enrichment/depletion is calculated as

$$\frac{C_{continent} - C_{origimal}}{C_{original}}$$

where $C_{continent}$ is the content of given residue in the query set of S proteins on a given continent and $C_{original}$ is the content of the same residue in the original S protein. For comparison, we generated composition profiles of disordered proteins, where normalized composition was evaluated as $\frac{C_{DisProt} - C_{PDB}}{C_{PDB}}$ ($C_{DisProt}$ is the content of a given amino acid in the set of intrinsically disordered proteins in the DisProt database [30]; C_{PDB} is the content of the given residue in the dataset of fully ordered proteins, PDB-Select-25 [29]). In these analyses, the positive and negative values produced in the compositional profiler indicated enrichment or depletion of the indicated residue, respectively.

2.4. Amino acid conservation Shannon entropy

How conserved/disordered the amino acids are organized in the S protein is addressed by the information-theoretic measure known as '*Shannon entropy*' (SE). For each S protein, Shannon entropy of amino acid conservation in the amino acid sequence of the S protein is computed using the following formula [31,32]:

For a given amino acid sequence of length l, the conservation of amino acids is calculated as follows:

$$SE = -\sum_{i=1}^{20} p_{s_i} log_{20}(p_{s_i})$$

where $p_{s_i} = \frac{k_i}{l}$; k_i represents the number of occurrences of an amino acid s_i in the given sequence [33].

2.5. Isoelectric point of a protein sequence

The isoelectric point (pI), is the pH at which a molecule carries no net electrical charge or is electrically neutral in the statistical mean. We calculated the theoretical pI by using the pKa's of amino acids and summing the net charge across the protein at a given pH (default is typical intracellular pH 7.2), searching with our algorithm for the pH at which the net charge is zero [34]. The isoelectric point is a powerful tool to predict and understand interactions between proteins, proteins and membranes or to determine the presence of protein isoforms [35]. Furthermore, it is noted that the isoelectric point is one of the prime keys for understanding a variety of biochemical properties of protein sequences [35,36]. Note that the isoelectric point of a protein sequence was computed here using the standard routine of Matlab-2021a. This parameter was deployed to characterize the unique S protein sequences, quantitatively.

2.6. Intrinsic disorder analysis

Intrinsic disorder predisposition of the S protein from the original (Wuhan) version of SARS-CoV-2 was analyzed by a set of six commonly used disorder predictors, such as PONDR® VLXT, PONDR® VL3, PONDR® VSL2B, PONDR® FIT, IUPred2 (Short) and IUPred2 (Long), which were selected for their specific features. The outputs of the evaluation of the per-residue disorder propensity by these tools are represented as real numbers between 1 (ideal prediction of disorder) and 0 (ideal prediction of order) [37–41]. Thresholds of $\geq$0.15 and $\geq$0.5 were used to identify flexible and disordered residues and regions.The intrinsic disorder profile of this protein was generated by DiSpi/RIDAO web-crawler that combines the outputs of PONDR® VLXT, PONDR® VL3, PONDR® VLS2B, PONDR® FIT, IUPred2 (Short) and IUPred2 (Long) on the one plot and complement them by the errors evaluated for the mean disorder profile calculated by averaging profiles of individual predictors. Analysis of intrinsic disorder predisposition of unique variants of the S protein was conducted by PONDR® VSL2B. This tool is commonly used in the analysis of disorder predisposition of proteins and systematically shows good performance in various comparative analyses, including the recently conducted Critical assessment of protein intrinsic disorder prediction (CAID) experiment, where PONDR® VLS2B was recognized as predictor #3 of the 43 evaluated methods [42].

3. Results

We first determined the set of unique S protein sequences from each continent. Further, every unique S protein from a continent was compared with other unique S proteins from five other continents, and the lists of the same are presented in Tables 12–17. Also, the variability of the S proteins from each continent was shown using Shannon entropy and isoelectric point.

3.1. Unique S proteins on the continents

In Table 1, the number of total sequences, unique sequences and percentages are presented. Note that, a complete list of unique S protein accessions and their names (continent-wise) are made available in Supplementary file-1. Note that, sequence accession is renamed as *Ck*

Table 1
Percentages of continent-wise unique spike (S) proteins.

Continent	Total S proteins (T)	Unique S proteins (U)	Percentage, continent-wise $\frac{U}{T} \times 100$	Percentage, worldwide $\frac{U}{16,143} \times 100$
Africa	984	286	29.065	1.772
Asia	2314	432	18.669	2.676
Europe	1006	187	18.588	1.158
Oceania	9920	1121	11.300	6.944
South America	464	71	15.302	0.440
North America	113,072	14,046	12.422	87.010
Worldwide	127,760	16,143	12.635	–

Table 2
The total continent-wise number of identical S proteins.

Continent-wise	Asia	Africa	Europe	North America	Oceania	South America
Asia	–	25	27	169	17	17
Africa	25	–	15	71	13	5
Europe	27	15	–	76	9	8
North America	169	71	76	–	49	31
Oceania	17	13	9	49	–	5
South America	17	5	8	31	5	–
Total continent-wise	255	129	135	396	93	66
Unique residue S proteins	177	157	52	13,650	1028	5

Table 3
The total number and percentage of invariant residue positions among 1273 positions in unique S proteins.

Frequency of invariant residue positions in unique S proteins from each continent						
	Africa	Asia	Europe	Oceania	South America	North America
Total freq.	902	695	948	731	1070	89
Percentage	70.86	54.60	74.47	57.42	84.05	6.99

where *C* stands for continent code (Asia: AS, Africa: AF, Oceania: O, Europe: U, South America: SA, and North America: NA), and *k* denotes the serial number.

The highest percentage (29.065%) of unique S proteins was found in Africa though the total number of available sequences is significantly lower as compared with that from other continents. Almost similar amounts (in percentage) of unique S sequence variations were found in Asia and Europe. Among the total 127,760 S proteins embedded in SARS-CoV-2 genomes, only 16,143 (12%) unique S proteins were detected so far, and notably most of the unique variants (87%) were found in North America only.

For each continent, the unique S proteins were matched with other unique proteins from the rest of the five continents, and a total number of such identical pairs are presented accordingly in the matrix (Table 2).

From Table 2, it was observed that, on each continent there is still a significant percentage of unique S variations available, which are not shared with any other continent. Such percentages of unique variations of S proteins in Asia, Africa, Europe, Oceania, South America, and North America were 41%, 55%, 28%, 92%, 7%, and 97% respectively. The lists of pairs of identical S proteins of SARS-CoV-2 originating from six continents are presented in Tables 9–11 (see Appendix A). The lists of unique S proteins (from a particular continent), which were found to be identical with some unique S proteins from the other five continents, are presented in Tables 12–17 (see Appendix A).

The frequency and percentage of invariant residue positions, where no amino acid change was detected so far in the unique S proteins available on each continent, are presented in Table 3.

The highest number of mutations (lowest number of invariant residue position, 6.99%) (Table 3) were detected in the unique S proteins from North America where 12.42% unique S protein sequences were present as mentioned in Table 1. Likewise, the lowest number (15.95%) of mutations in unique S proteins was observed in South America where 15.3% unique S sequences were found. Only 29.14% residues of 1273 in the unique S proteins were mutated, although a significantly higher number (29.065%) of unique sequences were found in Africa compared to the other five continents. The unique S proteins from Europe possessed only 25.5% mutations, whereas 45.5% mutations were detected in the unique S proteins from Asia, although the same percentage (18.5%) of unique S proteins were found (Tables 1 and 3). Further, it was observed that 11.3% of the unique S proteins from Oceania possessed 42.58% mutations.

3.2. Phylogenetic relationship among unique S protein variants

We collected 204,440 S protein sequences from NCBI and GAISED databases. Upon filtering, 191,536 redundant sequences were removed and 12,904 unique sequences (corresponding to 6.31% of the initial number of sequences) were selected for phylogenetic analysis.

The resultant phylogeny for unique amino acid sequences from the SARS-CoV-2 S protein, revealed a tree with polyphyletic groups, as well as showing sequences from different continents grouping together in the same clade (see Supplementary Fig. 1). On the other hand, after the Archaepteryx analysis five predominant sequence groups were identified between different S variants from different continents (Fig. 1).

In this case, it can be verified that the sequences from the same continents can be found in the groups with different colors. Therefore, this phylogenetic analysis grouped together sequences from different continents. Then, after these analyses it could be possible to assume that we have at least five unique SARS-CoV-2 S variants indicating possibilities for new ways of developing more specific vaccines and drugs.

3.3. Variability through normalized amino acid composition

Additional information on the variability of the amino compositions of the unique S proteins from each continent relative to the composition of the original S protein from Wuhan was retrieved using the web-based tool Composition Profiler (http://www.cprofiler.org/). Results of this analysis are shown in Fig. 2A, which clearly shows the presence of some noticeable amino acid composition variability among unique S proteins from different continents. Since individual S proteins are different from each other and from the original S protein mostly in very limited number of residues, the range of changes in the normalized enrichment/depletion of a given residue is rather limited (compare scales of Y axis in Fig. 2A and B, where a composition profile of the intrinsically disordered proteins is shown for comparison).

On an average, unique S proteins form Oceania were found to have the most variability in terms of normalized amino acid composition. This was followed by the unique S proteins from North America. Curiously, Fig. 2A shows that although the normalized content of individual residues in the unique S proteins from Oceania is always below that of the original S protein, S proteins from other continents might have a relative excess of some residues. For example, some unique S proteins from almost all continents can be enriched in glycine or histidine residues, whereas some European S proteins can also be relatively enriched in cysteine, isoleucine, tyrosine, phenylalanine, and lysine residues (see positive green bars in Fig. 2A). Another interesting observation is that the different sets of S proteins are typically characterized by rather noticeable variability of the normalized content of most residues. Aspartate is the noticeable exception, for which depletion is almost uniform between all unique S proteins from all continents.

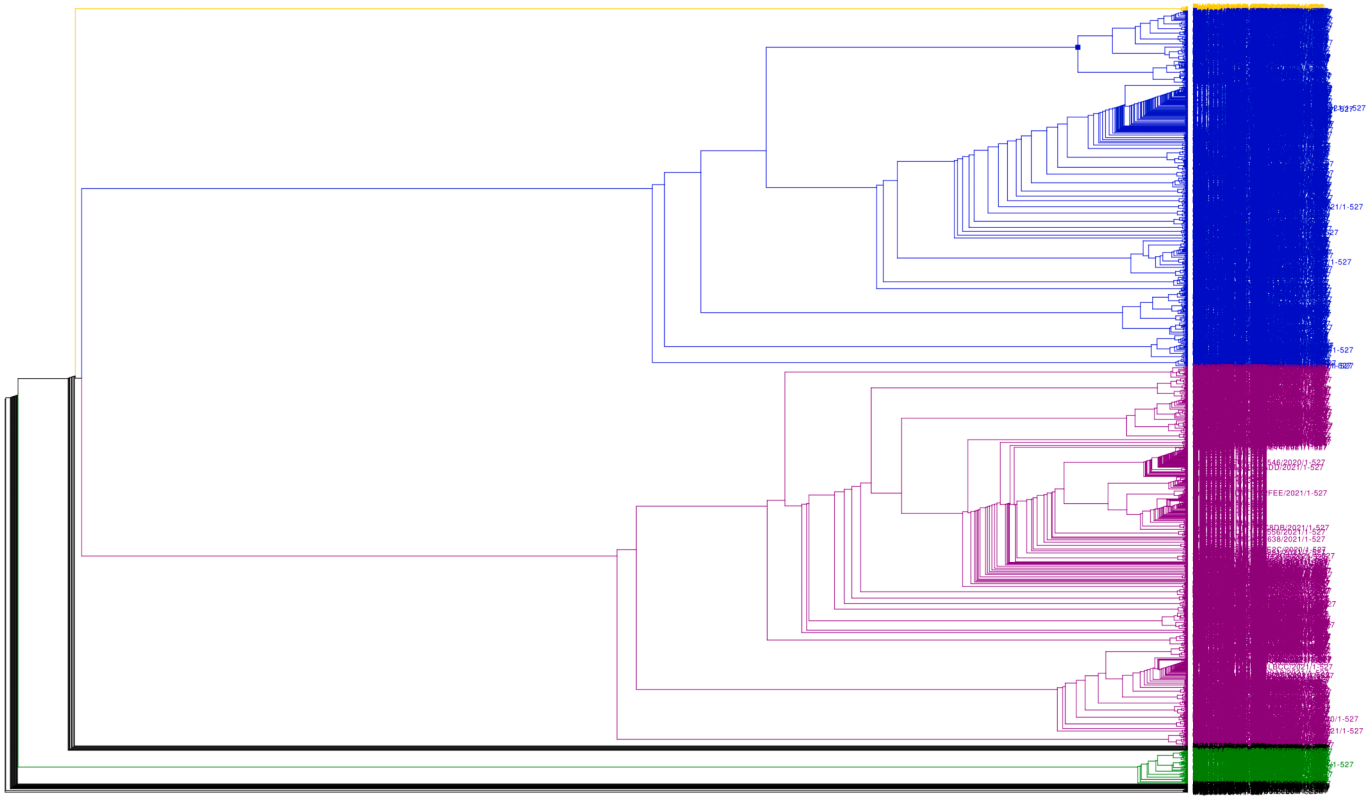

Fig. 1. SARS-CoV-2 S amino acid phylogeny after group clustering. After Archeopteryx analysis five groups can be identified: yellow, blue, magenta, green and black.

3.4. Variability through intrinsic disorder analysis

Next, we looked at the correlation between frequencies of the mutations in unique S proteins from different locations and intrinsic disorder predisposition of this protein. Fig. 3A shows the distribution of the mutation frequency within the amino acid sequence of the S protein. It is seen that almost all residues have at least one mutation in different variants currently found globally. In fact, only 15 residues (Met_1, Leu_{996}, Ile_{997}, Gly_{999}, Leu_{1001}, Tyr_1007, Val_1008, Gln_{1010}, Ile_{1013}, Arg_{1019}, His_{1049}, Gln_{1054}, Thr_{1105}, Asn_{1119}, and Leu_{1270}) of the 1273-residue long S protein showed no mutations at the time of this analysis. Curiously, nine of these never-changed residues are concentrated within a short region (residues 996-1019). Fig. 3A also shows that mutation frequencies are unevenly distributed within the amino acid sequence of the S protein and that the region (residues 675-691) surrounding the furin cleavage site (residues 680-686) seems to be characterized by high mutation frequency. In fact, although the average per-residue frequency of mutations of the entire protein is equal to 4.6, the mutation frequency of the 675-691 region is two-fold higher (9.2). Comparison of the mutation frequency profile (Fig. 3A) with the per-residue intrinsic disorder predisposition profile generated for the original (Wuhan) version of the S protein by a set of commonly used disorder predictors (Fig. 3B) indicated that there is some weak correlation between these two parameters, with regions showing more disorder typically are undergoing more frequent mutations. Again, Fig. 3B shows that the region containing the furin cleavage site is among the most disordered segments of the S protein (if not the most disordered one).

Fig. 4 provides further quantification of the per-residue mutability and disorder predisposition of the S protein. Here, dependencies of the mutation frequencies on the corresponding disorder score evaluated by PONDR® VSL2 are shown for six geo-locations. In Africa, the S protein has 902, 296, 60, 11, and 4 residues with 0, 1, 2, 3, and 4 mutations, which are characterized by the mean disorder scores of 0.27 ± 0.15, 0.28 ± 0.16, 0.30 ± 0.18, 0.29 ± 0.18, and 0.40 ± 0.19, respectively. In Asia, 694, 437, 111, 27, 3, and 1 residues of the S protein with 0, 1, 2, 3, 4, and 5 mutations are characterized by the mean disorder scores of 0.26 ± 0.14, 0.28 ± 0.15, 0.32 ± 0.16, 0.37 ± 0.18, 0.52 ± 0.22, and 0.19, respectively. In Europe, 948, 265, 55, and 5 residues of the S protein with 0, 1, 2, and 3 mutations have the mean disorder scores of 0.27 ± 0.15, 0.28 ± 0.15, 0.33 ± 0.18 and 0.35 ± 0.27, respectively. The S proteins in Oceania has 722, 427, 107, 13, and 4 residues with 0, 1, 2, 3, and 4 mutations, which are showing the disorder scores of 0.26 ± 0.14, 0.28 ± 0.16, 0.30 ± 0.17, 0.35 ± 0.21, and 0.27 ± 0.15, respectively. In the S proteins from South America variants, 1070, 193, and 10 residues with 0, 1, and 2 mutations have mean disorder scores of 0.27 ± 0.15, 0.36 ± 0.13, and 0.23 ± 0.10, respectively. Finally, in North America, the S protein underwent most mutations and has 23, 351, 323, 234, 167, 108, 42, 12, 6, 4, 1, 1, and 1 residues with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 13 mutations characterized by the mean disorder scores of 0.28 ± 0.17, 0.25 ± 0.13, 0.25 ± 0.13, 0.29 ± 0.17, 0.31 ± 0.19, 0.38 ± 0.16, 0.4 ± 0.17, 0.22 ± 0.16, 0.42 ± 0.22, 0.18, 0.25, and 0.45, respectively.

The most frequently mutated residue is Tyr_{248} (13 mutations) followed by Val_{213} and Thr_{108} with 12 and 10 mutations, respectively, all three from unique S protein variants found in North America. This analysis shows that here is a general trend, where residues with higher disorder levels are mutated more frequently.

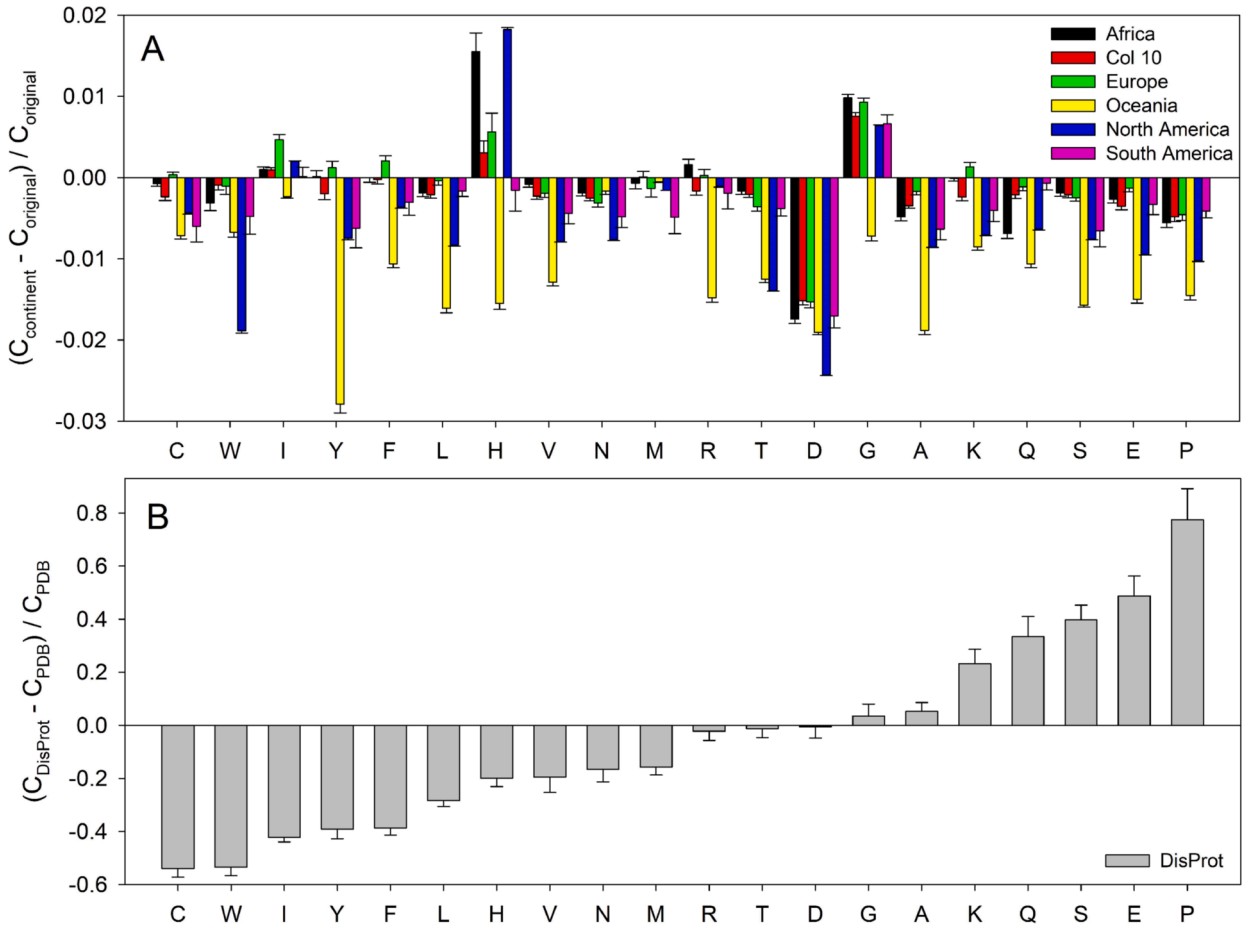

Fig. 2. Composition profiles of unique S proteins from different continents (A) in comparison with the composition profile of typical intrinsically disordered proteins (B). (For interpretation of the references of the colors in this figure, the reader is referred to the web version of this article.)

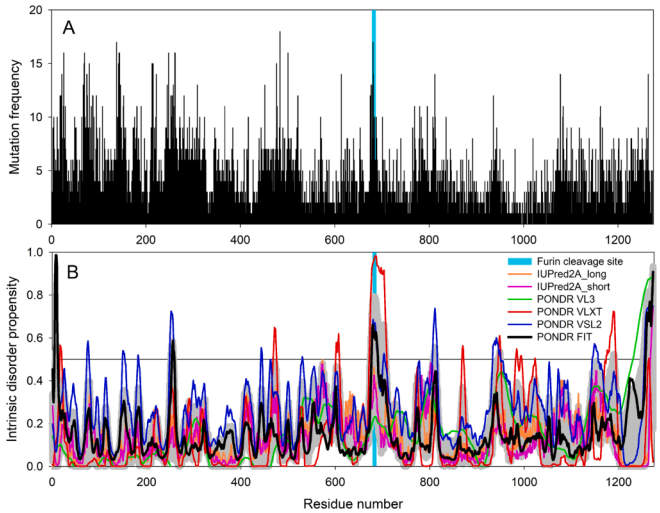

Fig. 3. Correlation of the sequence variability of unique variants of the S protein with the intrinsic disorder predisposition of this protein. A. Mutation frequencies observed at each residue of the S protein at various locations. B. Intrinsic disorder predisposition of the original (Wuhan) version of the S protein analyzed by a set of commonly used disorder predictors. In both plots, the position of the furin cleavage site is shown as a cyan vertical bar. (For interpretation of the references of the colors in this figure legend, the reader is referred to the web version of this article.)

3.5. *Variability of unique S proteins*

We quantitatively determined the variations in the unique S proteins on six continents. The variations were captured through the frequency distribution of amino acids present, Shannon entropy (amount of conservation of amino acids in a given sequence), and molecular weight and isoelectric point of a given protein sequence.

3.5.1. *Variations in the frequency distribution of amino acids*

The frequency of each amino acid was computed for each unique S protein available on six continents (Supplementary file-2). Maximum and minimum frequencies of amino acids present in the unique S proteins from different continents are presented in Table 4.

All S protein sequences are leucine (L) and serine (S) rich. Tryptophan (W) and methionine (M) were presented with the least frequencies (Table 4). The widest variation in frequency distributions of the twenty amino acids in the unique S proteins was found in North America.

To obtain quantitative variations in the unique S proteins available on each continent, differences between maximum and minimum vectors (20 dimensions) were obtained (Table 5), and then Euclidean distances between the difference vectors were calculated (Table 6).

Based on the distance matrix, a phylogenetic relationship was derived among the continents (Fig. 2).

Variations based on the frequency distribution of amino acids present in the S proteins make North America (which belongs to the rightmost branch of the tree) distant from the other five continents

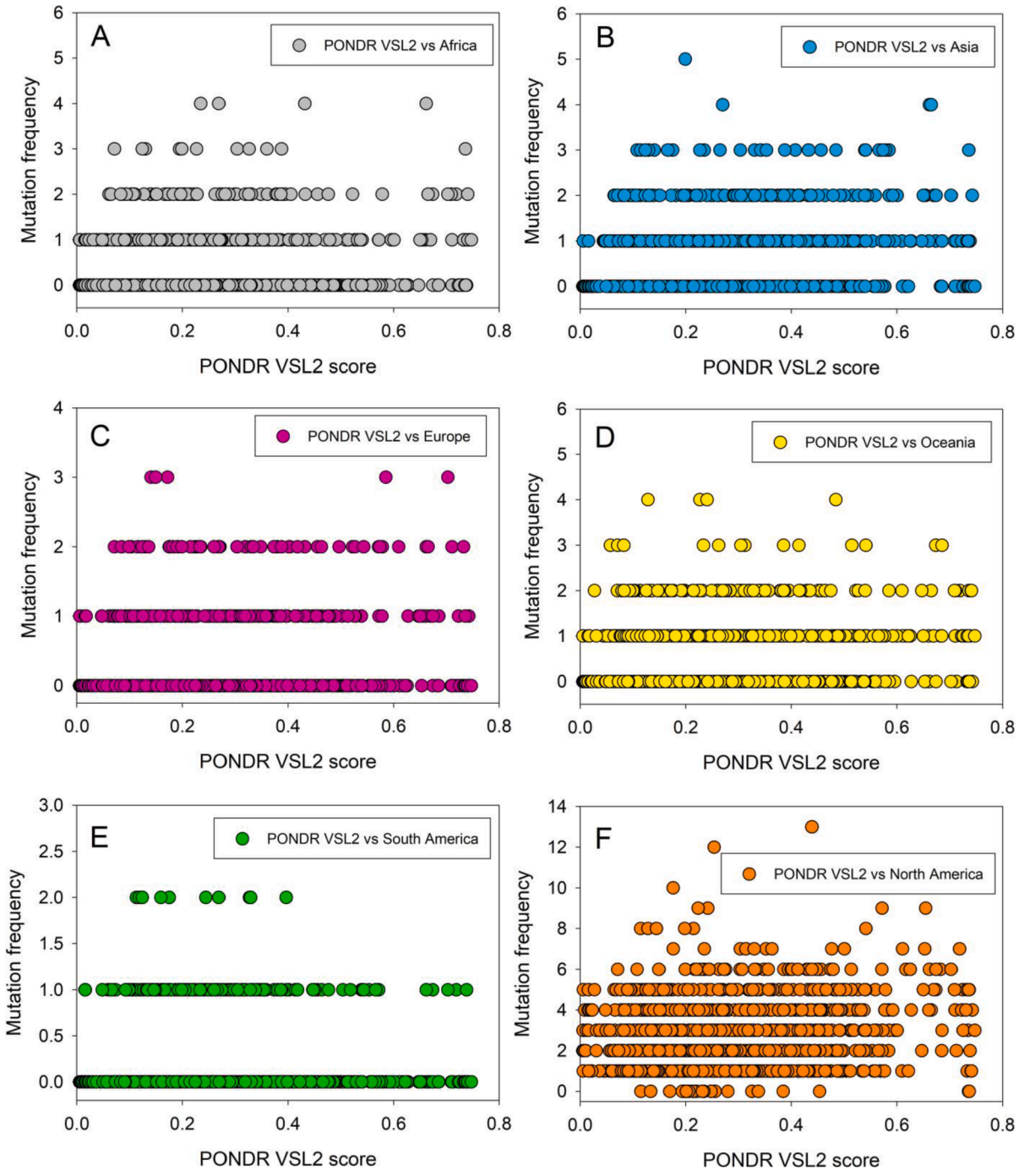

Fig. 4. Correlation of frequency of amino acid substitutions at a given residue of the S protein and the corresponding intrinsic disorder score of these residues within the sequence of the original (Wuhan) version of the S protein. Individual plots reflect distributions within sequences of variants found at different locations: A. Africa; B. Asia; C. Europe, D. Oceania, E. South America; F. North America.

(Fig. 2). Variations among the unique S proteins from Asia and Oceania turned out to be similar, and they belong to the same level of leaves of the far left branch of the tree. Africa and Europe were found to be the closest in terms of variations based on the frequency distribution of amino acids over the unique S proteins from each continent. Variability of S proteins from South America has distant resemblance to that of Africa/Europe as estimated in the phylogeny. The frequencies of amino acid distribution in each unique S protein from each continent are presented in Figs. 6 and 7 (see Appendix A). The widest variations of the frequency distribution of amino acids present in the S proteins were

Table 4
Maximum and minimum frequencies of amino acids present in the unique spike proteins from different continents.

Max and min of frequencies		A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
Africa	Max	80	44	89	62	41	63	49	84	19	79	109	62	15	78	60	101	98	13	56	98
	Min	73	40	85	58	38	59	45	78	14	73	102	57	13	72	55	94	90	11	49	93
Asia	Max	80	44	89	63	41	63	49	84	19	78	110	62	15	79	59	101	101	13	57	98
	Min	73	39	80	55	36	56	45	76	15	72	100	55	13	68	52	90	90	11	49	90
Europe	Max	80	43	89	63	41	63	49	84	19	79	110	62	15	79	59	101	98	13	57	99
	Min	75	38	84	59	39	59	46	79	16	74	102	58	13	74	54	96	90	11	50	93
Oceania	Max	81	43	90	62	41	63	49	84	18	78	109	62	15	79	59	100	98	12	56	99
	Min	72	37	81	58	36	57	44	74	15	71	97	56	13	71	52	92	88	10	43	89
South America	Max	82	44	91	63	42	64	49	85	20	79	111	64	15	80	60	102	99	13	58	100
	Min	60	32	63	46	32	39	34	63	11	55	82	43	9	55	43	76	77	8	36	82
North America	Max	80	43	89	62	41	63	48	83	18	78	109	62	14	79	58	101	98	12	57	98
	Min	75	38	82	57	37	59	45	79	16	73	105	57	13	73	57	92	93	11	50	92

Table 5
Matrix presenting the difference between maximum and minimum frequencies of amino acids present in the unique S proteins on each continent.

Difference matrix	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
Africa	7	4	4	4	3	4	4	6	5	6	7	5	2	6	5	7	8	2	7	5
Asia	7	5	9	8	5	7	4	8	4	6	10	7	2	11	7	11	11	2	8	8
Europe	5	5	5	4	2	4	3	5	3	5	8	4	2	5	5	5	8	2	7	6
Oceania	9	6	9	4	5	6	5	10	3	7	12	6	2	8	7	8	10	2	13	10
South America	5	5	7	5	4	4	3	4	2	5	4	5	1	6	1	9	5	1	7	6
North America	22	12	28	17	10	25	15	22	9	24	29	21	6	25	17	26	22	5	22	18

Table 6
Pairwise Euclidean distances among the differences in vectors of each continent.

Distance matrix	Africa	Asia	Europe	Oceania	South America	North America
Africa	0.00	11.70	4.69	12.77	8.49	66.80
Asia	11.70	0.00	13.00	9.06	14.04	57.02
Europe	4.69	13.00	0.00	13.30	8.49	68.38
Oceania	12.77	9.06	13.30	0.00	16.03	56.84
South America	8.49	14.04	8.49	16.03	0.00	69.02
North America	66.80	57.02	68.38	56.84	69.02	0.00

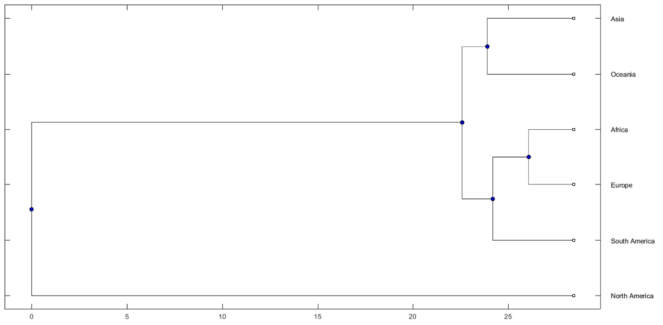

Fig. 5. Phylogenetic relationship among the six continents based on the variability of unique spike proteins available in each continent.

observed in North America as wide band was observed in Fig. 7. Individual frequency distributions of amino acids in Asia and Oceania seem very close as it was observed from the phylogeny (Fig. 5).

Table 7
Interval of Shannon entropy of the unique S proteins from six different continents.

SE: Continent	Interval of SEs
SE of S protein: Africa	(0.960825, 0.963239)
SE of S protein: Asia	(0.961471, 0.963326)
SE of S protein: Europe	(0.961539, 0.963254)
SE of S protein: North America	(0.95934, 0.964314)
SE of S protein: Oceania	(0.961525, 0.963042)
SE of S protein: South America	(0.961589, 0.962895)

Table 8
Interval of isoelectric point of unique S proteins from six different continents.

pI: Continent	Interval of PIs
pI of S protein: Africa	(6.44, 7.09)
pI of S protein: Asia	(6.21, 7.08)
pI of S protein: Europe	(6.21, 6.99)
pI of S protein: North America	(5.61, 7.79)
pI of S protein: Oceania	(6.31, 7.09)
pI of S protein: South America	(6.36, 6.99)

3.5.2. *Variability through Shannon entropy*

In principle, for a random amino acid sequence, the Shannon entropy (SE) is one. Here Shannon entropy for each S protein sequence was computed using the formula stated in Section 2.2 (Supplementary file-2). It was found that the highest and lowest SEs of the S proteins from all continents were 0.9643 and 0.9594 respectively. That is, the length of the largest interval is 0.005 which is sufficiently small. Also note that the length of the smallest interval was 0.001 which occurred in the SEs of the S proteins from South America. Within this realm, the widest

variation of SEs was noticed among the unique S proteins of North America. All other four intervals (considering lowest and highest) of SEs of all the unique S proteins from four continents Africa, Asia, Oceania and Europe were contained in the interval of North America and that of South America (Table 7).

Among all (20^{1273}) possible amino acids (20 in number) sequences of length 1273, nature had selected only a fraction to make the S proteins of SARS-CoV-2, and interestingly SEs of them were kept within a very small interval. From the SEs which were close to 1, the S protein sequences are expected to be pseudo-random. Variation of SEs for all unique S proteins from each continent is shown in Figs. 5 and 6 (see Appendix A). Conservation of amino acids present over each S protein from each continent is different from one another, which is depicted by the zig-zag nature of the SE plots (Figs. 8 and 9).

3.5.3. *Variability through isoelectric point*

For each S protein sequence from each continent the isoelectric point (pI) was computed (Supplementary file-3). Intervals (considering minimum and maximum) pIs of the unique S proteins from each continent are presented in Table 8.

It was noticed that pIs for all the unique S proteins from the six continents were distributed between 5.61 and 7.79. The largest interval of pIs was found for the unique S proteins from North America. Therefore, the widest varieties of the unique S proteins were found in North America.

The degree of non-linearity of the plots of pIs for each protein from each continent shows wide variations of the unique S proteins (Figs. 10 and 11 (see Appendix A)).

4. Discussion and concluding remarks

Various mutations in the S proteins lead to the evolution of new variants of SARS-CoV-2 [43]. Naturally, our attention was captured to characterize the unique S protein variants, which were embedded in SARS-CoV-2 genomes infecting millions of people worldwide [44]. As of May 7, 2021, there were 127,760 COVID-19 patients infected with SARS-CoV-2 with 16,143 S protein variants, which are undoubtedly well-organized by means of amino acid composition and conservation as depicted by Shannon entropy and isoelectric point. Among the unique S proteins present on a continent, many of them are common on other continents as well (Table 2). On the other hand, there are still a handful of unique S protein variants residing on each continent. Considering the nature and biological implications of the new variants of SARS-CoV-2 caused by different mutations in the S proteins, the appearance of several unique S variants in SARS-CoV-2 is certainly a worrying trend [45]. There are still many unique S protein variants on all continents that may spread from person to person through close communities or by spontaneous mutations causing a condition that may become alarming.

Comparative analysis revealed the presence of some weak correlation between the per-residue mutability and intrinsic disorder predisposition of the S protein, with residues possessing higher disorder predisposition typically showing higher mutation rates as well. For example, the mean disorder score of 89 residues that were mutated 10-18 times is 0.35 ± 0.18 as compared to the mean disorder score of 0.26 ± 0.12 for 155 residues with 0 and 1 mutations. Curiously, the most disorder region of the S protein (residues 675-691), which includes the furin cleavage site (residues 680-686), was shown to be characterized by high mutation frequency, with Pro_{681} (which was mutated 17 times) being the second most frequently mutated residue of this protein.

We observed that the unique S proteins from North America have mutations in almost every amino acid residue position (1184 out of 1273), while unique S variants from the other continents only have mutations in 16 to 20% of residues. So, even if international travel is limited, S proteins from these five continents will likely acquire mutations at other residue positions where mutations have already been found in the specific variants from North America due to natural evolution. Based on the amino acid frequency distributions in the S protein variants from all continents, a phylogenetic relationship among the continents was presented. The phylogenetic relationship implies that the unique S proteins from North America were found to be significantly different from those of the other five continents. Therefore, the possibility of spreading the unique variants originating from North America to the other geographic locations by means of international travel is high, and numerous mutations have been detected already in the unique variants from North America. Of note, the infection/herd immunity status in South America may be summarized by the example of Manaus (the capital of Amazonas state in northern Brazil) where from June 2020 to October 2020 the SARS-CoV-2 prevalence among the population increased from ~60% to ~70%, a condition which may mirror acquisition of herd immunity [46]. By January 2021 Manaus had a huge resurgence in cases due to emergence of a new variant known as P.1, which was responsible for nearly 100% of the new cases [47]. Although the population may have then reached a high herd immunity threshold, there is still a risk of resurgence of new immunity-escape variants, which raises important questions. For example, 1. Is post-infection herd immunity not enough for protection and should it be combined with vaccinations? 2. Will the crucial viral variants (mutations) be listed by the WHO and recommended to be included in “next generation vaccines”? [48,49]. In addition, we cannot yet exclude the possibility of detrimental mutations in the viral Spike-RBD emerging in India and the USA [48].

Let us have a brief glance at the potential consequences of the mutations in S-protein from the viewpoint of protective immunity towards SARS-CoV-2. It is known that the protective immunity towards infection and disease depends on the presence of high avidity antibodies. This is because high avidity of neutralizing antibodies, which is defined as the strength of antibody-target epitope interaction, plays an important role in antibody-mediated protection against viral infections [12]. High avidity (functional affinity) is established during affinity maturation, as the avidity of IgG is low during acute infection and reaches high values several weeks or months later [50,51]. Importantly, incomplete avidity maturation of IgG often leads to the failure of the protection against viral infections and/or resultant diseases, as was shown for varicella zoster virus (VZV), cytomegalovirus (CMV), measles virus, Dengue virus, respiratory syncytial virus (RSV), and Simian human immunodeficiency virus (SHIDV) [52–59]. Since the interaction between the SARS-CoV-2 Spike-RBD protein and ACE2 on host cells is characterized by high affinity, it is expected that the protective anti-SARS-CoV-2 antibodies should possess high affinity/avidity to be able to block this high affinity RBD-ACE2 interaction [11]. Recently, it was shown that the serological response to SARS-CoV-2 is frequently characterized by the incomplete maturation of avidity [60,50]. It was also proposed that such incomplete avidity maturation represents an essential strategy of coronaviruses determining high probability of repeated waves of reinfections due to the short-lasting protective immunity [61,62,12]. Furthermore, an unexpected scenario was recently uncovered, where the natural SARS-CoV-2 infection does not lead to the establishment of a high avidity immune response and therefore does not have a good chance for the development of complete protection against SARS-CoV-2 and for establishment of

herd immunity [63]. On the contrary, complete avidity maturation was achieved with two rounds of vaccination, whereas the quality of the immune response after natural infection was similar to that generated by one vaccination step and did not reach the quality of complete vaccination with two steps. Therefore, the scenario occurring in Manaus can be considered on the basis of these new findings. In fact, it is obvious now that despite the high COVID-19 prevalence reached in this city, no herd immunity could be expected retrospectively, as natural infections are insufficient for the establishment of a high avidity immune response and related development of complete protection against SARS-CoV-2 [63]. Therefore, it seems likely that the herd immunity can only be reached through at least two vaccination steps [63]. It is also expected that herd immunity might be partially or completely overrun by novel SARS-CoV-2 variants showing higher affinity of RBD-ACE2 interaction than that of the original SARS-CoV-2 strain.

Hence in the near future, we can expect to experience more new SARS-CoV-2 variants, which might cause additional. waves of COVID-19. Therefore, massive vaccination is necessary to combat COVID-19, and of course, existing vaccines must be reviewed, and if needed further re-engineered based on newly emerging S protein variants.

Altogether, data presented in this study indicate that although unique variants of the SARS-CoV-2 S protein are rather abundant, they are unevenly distributed among continents, with Africa possessing the highest percentage of unique S variants, and with the unique S proteins found in North America being noticeably different from the variants on other continents. It is likely that these unique variants can spread to continents where they have not been detected before. Furthermore, this inhomogeneity raises an important question on why the currently observed differences in the number of unique variants of the S protein (reflecting frequency of its mutagenesis) is so great. It cannot be easily explained by the differences between the continents in the number of COVID-19 patients (reported SARS-CoV-2-positive cases). In fact, according to Worldometer, as of September 10, 2021, there were 8,087,058, 72,407,564, 56,618,705, 181,742, 50,106,216, and 37,213,429 recorded COVID-19 cases in Africa, Asia, Europe, Oceania, North America, and South America, respectively. Obviously, these infection levels do not correlate with the corresponding numbers of unique S protein variants (see Tables 1 and 2). There is also no strong correlation between the reported S protein variability and levels of genomic sequencing on different continents (which serves now as a real-time molecular/genomics SARS-CoV-2 surveillance). In fact, it was reported that as of 5 July 2021, 25,284 whole-genome sequences from Africa (0.32% of all reported SARS-CoV-2-positive cases from that continent), 146,562 from Asia (0.30% coverage), 1,292,415 from Europe (2.35% coverage), 692,704 from North America (1.75% coverage), 37,913 from South America (0.12% coverage) and 20,613 from Oceania (25% coverage) had been generated [64]. Therefore, although these numbers reflecting levels of the continent-wise coverage show a heavy bias towards the regions and countries with more specialized genomics facilities, programs, and research projects, there is no strong correlation between the coverage and established S-protein variability [65,66].

An intriguing possible mechanism of the observed differences in the rates of virus evolution is the presence of a conceivable variability of the ACE2 gene on different continents that might have an impact on the variability of the viral protein as well. In line with this idea, it was recently reported that the expression levels of ACE2 can be elevated up to 50% due to the differences in the frequency of the rs2285666 polymorphism (the TT-plus strand or AA-minus strand alternate allele) among Europeans and Asians, with this difference playing a significant role in the SARS-CoV-2 susceptibility [67,68]. Similarly, based on comprehensive analyses of the allelic frequencies of polymorphisms in the ACE2, TMPRSS2, TMPRSS11A, cathepsin L (CTSL), and elastase (ELANE) genes in populations from the American, African, European, and Asian continents it was concluded that the non-coding sequences of these proteins related to the SARS-CoV-2 cell entry contain numerous polymorphisms with possible functional consequences [69].

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2021.09.080.

CRediT authorship contribution statement

Sk. Sarif Hassan: Study design, Formal analysis, Investigation, Methodology, Visualization, Writing - Original draft, Writing - Review & editing, Administration;
Kenneth Lundstrom: Formal analysis, Investigation, Writing - Original draft, Writing - Review & editing;
Debmalya Barh: Formal analysis, Investigation, Methodology, Visualization, Writing - Review & editing;
Raner Jośe Santana Silva: Formal analysis, Investigation, Methodology, Visualization, Writing - Review & editing;
Bruno Silva Andrade: Formal analysis, Investigation, Methodology, Visualization, Writing - Review & editing;
Vasco Azevedo: Formal analysis, Investigation, Methodology, Visualization, Writing - Review & editing;
Pabitra Pal Choudhury: Formal analysis, Writing - Review & editing;
Giorgio Palu: Formal analysis, Writing - Review & editing;
Bruce D. Uhal: Formal analysis, Writing - Review & editing;
Ramesh Kandimalla: Formal analysis, Writing - Review & editing;
Murat Seyran: Formal analysis, Writing - Review & editing;
Amos Lal: Formal analysis, Writing - Review & editing;
Samendra P. Sherchan: Formal analysis, Writing - Review & editing;
Gajendra Kumar Azad: Formal analysis, Writing - Review & editing;
Alaa A. A. Aljabali: Formal analysis, Writing - Review & editing;
Adam M. Brufsky: Formal analysis, Writing - Review & editing;
Angel Serrano-Aroca: Formal analysis, Writing - Review & editing;
Parise Adadi: Formal analysis, Writing - Review & editing;
Tarek Mohamed Abd El-Aziz: Formal analysis, Writing - Review & editing;
Elrashdy M. Redwan: Formal analysis, Writing - Review & editing;
Kazuo Takayama: Formal analysis, Writing - Review & editing;
Nima Rezaei: Formal analysis, Writing - Review & editing;
Murtaza Tambuwala: Formal analysis, Writing - Review & editing;
Vladimir N. Uversky: Study design, Formal analysis, Methodology, Investigation, Visualization, Writing - Original draft, Writing - Review & editing.

All authors read and approved the final version of the manuscript.

Declaration of competing interest

Authors have no conflict of interest to declare.

Appendix A

Table 9
List of pairs of identical spike proteins of SARS-CoV-2 originated from six continents.

Spike: Asia-Europe	Spike: Asia-Africa	Spike: Asia-Oceania	Spike: Asia-South America	Spike: Asia-North America
(A14, U2)	(A14, AF2)	(A15, O5)	(A31, SA1)	(A1, NA7)
(A15, U3)	(A15, AF3)	(A77, O43)	(A67, SA4)	(A8, NA231)
(A30, U8)	(A26, AF19)	(A95, O58)	(A148, SA13)	(A12, NA902)
(A31, U9)	(A71, AF48)	(A109, O83)	(A180, SA19)	(A14, NA928)
(A33, U11)	(A93, AF58)	(A128, O201)	(A191, SA22)	(A15, NA992)
(A36, U17)	(A128, AF72)	(A138, O370)	(A200, SA25)	(A19, NA1131)
(A43, U18)	(A138, AF76)	(A142, O373)	(A207, SA27)	(A23, NA1445)
(A69, U23)	(A142, AF79)	(A148, O377)	(A211, SA30)	(A28, NA2065)
(A77, U26)	(A148, AF82)	(A166, O387)	(A213, SA32)	(A30, NA3228)
(A93, U28)	(A161, AF88)	(A206, O388)	(A219, SA33)	(A31, NA3313)
(A95, U30)	(A164, AF92)	(A213, O390)	(A234, SA35)	(A32, NA3438)
(A105, U34)	(A166, AF101)	(A253, O398)	(A280, SA41)	(A33, NA3477)
(A128, U52)	(A191, AF115)	(A277, O400)	(A284, SA42)	(A34, NA3658)
(A134, U54)	(A206, AF118)	(A284, O402)	(A335, SA61)	(A43, NA3752)
(A135, U57)	(A213, AF120)	(A305, O504)	(A340, SA63)	(A44, NA3768)
(A148, U63)	(A275, AF130)	(A359, O1076)	(A373, SA68)	(A58, NA3911)
(A213, U80)	(A276, AF131)	(A404, O1104)	(A404, SA71)	(A69, NA4028)
(A234, U84)	(A277, AF134)			(A71, NA4051)
(A239, U88)	(A279, AF137)			(A76, NA4169)
(A265, U94)	(A282, AF138)			(A77, NA4243)
(A284, U99)	(A292, AF147)			(A78, NA4270)
(A286, U100)	(A379, AF229)			(A85, NA4296)
(A333, U121)	(A394, AF247)			(A89, NA4375)
(A340, U124)	(A404, AF263)			(A90, NA4394)
(A379, U151)	(A430, AF278)			(A91, NA4436)
(A404, U181)				(A93, NA4448)
(A430, U187)				(A95, NA4508)
Spike: Asia-North America	Spike: Asia-North America	Spike: Asia-North America	Spike: Asia-North America	Spike: Asia-North America
(A96, NA4537)	(A166, NA5819)	(A214, NA6445)	(A267, NA6903)	(A345, NA9597)
(A97, NA4541)	(A170, NA5927)	(A215, NA6465)	(A273, NA6916)	(A348, NA9612)
(A100, NA4559)	(A171, NA5977)	(A216, NA6492)	(A274, NA6936)	(A351, NA9663)
(A101, NA4620)	(A173, NA5992)	(A217, NA6499)	(A275, NA6944)	(A354, NA9674)
(A102, NA4637)	(A174, NA6060)	(A218, NA6510)	(A276, NA6949)	(A356, NA9724)
(A103, NA4658)	(A175, NA6067)	(A219, NA6515)	(A277, NA6962)	(A357, NA9763)
(A105, NA4715)	(A177, NA6071)	(A221, NA6527)	(A278, NA6969)	(A358, NA9776)
(A109, NA4861)	(A178, NA6080)	(A222, NA6540)	(A279, NA7000)	(A359, NA9792)
(A111, NA4897)	(A180, NA6101)	(A223, NA6550)	(A280, NA7015)	(A360, NA9834)
(A114, NA5001)	(A181, NA6142)	(A224, NA6553)	(A282, NA7025)	(A367, NA10276)
(A115, NA5022)	(A182, NA6148)	(A230, NA6602)	(A283, NA7056)	(A373, NA10342)
(A121, NA5105)	(A183, NA6155)	(A233, NA6616)	(A284, NA7090)	(A375, NA10442)
(A122, NA5137)	(A191, NA6185)	(A234, NA6622)	(A286, NA7129)	(A378, NA11135)
(A126, NA5151)	(A193, NA6193)	(A235, NA6630)	(A291, NA7198)	(A379, NA11225)
(A127, NA5182)	(A195, NA6244)	(A238, NA6659)	(A292, NA7227)	(A380, NA11305)
(A128, NA5194)	(A196, NA6258)	(A239, NA6661)	(A293, NA7249)	(A381, NA11560)
(A133, NA5471)	(A198, NA6276)	(A244, NA6683)	(A304, NA7576)	(A383, NA11874)
(A134, NA5485)	(A199, NA6293)	(A245, NA6687)	(A322, NA8509)	(A386, NA13280)
(A135, NA5516)	(A200, NA6299)	(A247, NA6707)	(A323, NA8519)	(A387, NA13307)
(A138, NA5538)	(A201, NA6305)	(A249, NA6713)	(A324, NA8565)	(A388, NA13362)
(A140, NA5574)	(A205, NA6324)	(A253, NA6751)	(A325, NA8570)	(A391, NA13404)
(A148, NA5595)	(A206, NA6334)	(A254, NA6756)	(A333, NA9283)	(A394, NA13438)
(A158, NA5644)	(A207, NA6373)	(A255, NA6780)	(A335, NA9324)	(A395, NA13444)
(A159, NA5645)	(A210, NA6388)	(A257, NA6794)	(A341, NA9425)	(A396, NA13465)
(A161, NA5666)	(A211, NA6406)	(A258, NA6810)	(A342, NA9455)	(A399, NA13554)
(A163, NA5722)	(A212, NA6424)	(A264, NA6857)	(A343, NA9568)	(A401, NA13614)
(A164, NA5744)	(A213, NA6429)	(A265, NA6862)	(A344, NA9592)	(A404, NA13635)
				(A405, NA13668)
				(A408, NA13704)
				(A413, NA13841)
				(A418, NA13913)
				(A419, NA13948)
				(A430, NA14000)
				(A431, NA14026)

Table 10
List of pairs of identical spike proteins of SARS-CoV-2 originated from different continents.

Spike: Africa-Europe	Spike: Africa-North America	Spike: Africa-North America	Spike: Africa-Oceania	Spike: Africa-South America	Spike: Europe-North America
(AF2, U2)	(AF2, NA928)	(AF121, NA6566)	(AF1, O3)	(AF82, SA13)	(U2, NA928)
(AF3, U3)	(AF3, NA992)	(AF123, NA6628)	(AF3, O5)	(AF115, SA22)	(U3, NA992)
(AF31, U10)	(AF8, NA1298)	(AF125, NA6816)	(AF71, O148)	(AF117, SA26)	(U4, NA1221)
(AF58, U28)	(AF9, NA1348)	(AF128, NA6848)	(AF72, O201)	(AF120, SA32)	(U7, NA2680)
(AF69, U45)	(AF31, NA3387)	(AF130, NA6944)	(AF76, O370)	(AF263, SA71)	(U8, NA3228)
(AF72, U52)	(AF34, NA3583)	(AF131, NA6949)	(AF79, O373)		(U9, NA3313)
(AF82, U63)	(AF38, NA3797)	(AF133, NA6953)	(AF82, O377)		(U10, NA3387)
(AF120, U80)	(AF46, NA3986)	(AF134, NA6962)	(AF101, O387)		(U11, NA3477)
(AF123, U85)	(AF47, NA3988)	(AF137, NA7000)	(AF118, O388)		(U18, NA3752)
(AF145, U103)	(AF48, NA4051)	(AF138, NA7025)	(AF120, O390)		(U22, NA3895)
(AF195, U119)	(AF50, NA4061)	(AF145, NA7199)	(AF134, O400)		(U23, NA4028)
(AF229, U151)	(AF51, NA4117)	(AF146, NA7224)	(AF179, O751)		(U26, NA4243)
(AF230, U154)	(AF58, NA4448)	(AF147, NA7227)	(AF263, O1104)		(U28, NA4448)
(AF263, U181)	(AF64, NA4832)	(AF149, NA7286)		Spike: Oceania-South America	(U30, NA4508)
(AF278, U187)	(AF69, NA5149)	(AF151, NA7299)		(O377, SA13)	(U34, NA4715)
	(AF71, NA5188)	(AF152, NA7300)		(O389, SA28)	(U36, NA4780)
	(AF72, NA5194)	(AF154, NA7375)		(O390, SA32)	(U38, NA4837)
	(AF73, NA5202)	(AF156, NA7453)		(O402, SA42)	(U41, NA4989)
	(AF76, NA5538)	(AF165, NA7553)		(O1104, SA71)	(U42, NA5083)
	(AF82, NA5595)	(AF168, NA7644)			(U45, NA5149)
	(AF83, NA5606)	(AF179, NA8514)			(U47, NA5167)
	(AF88, NA5666)	(AF195, NA9264)			(U52, NA5194)
	(AF90, NA5693)	(AF196, NA9265)			(U53, NA5282)
	(AF92, NA5744)	(AF223, NA10257)			(U54, NA5485)
	(AF99, NA5818)	(AF227, NA10943)			(U55, NA5490)
	(AF101, NA5819)	(AF229, NA11225)			(U57, NA5516)
	(AF103, NA5829)	(AF230, NA11456)			(U63, NA5595)
	(AF104, NA5830)	(AF231, NA11576)			(U66, NA5627)
	(AF105, NA5837)	(AF247, NA13438)			(U72, NA6096)
	(AF108, NA5874)	(AF248, NA13478)			(U76, NA6240)
	(AF114, NA6178)	(AF254, NA13578)			(U78, NA6399)
	(AF115, NA6185)	(AF263, NA13635)			(U79, NA6421)
	(AF118, NA6334)	(AF268, NA13798)			(U80, NA6429)
	(AF119, NA6390)	(AF271, NA13870)			(U82, NA6450)
	(AF120, NA6429)	(AF278, NA14000)			(U84, NA6622)
		(AF283, NA14015)			(U85, NA6628)
					(U88, NA6661)
					(U90, NA6704)

Table 11
List of pairs of identical spike proteins of SARS-CoV-2 originated from different continents.

Spike: Europe-North America	Spike: Europe-Oceania	Spike: North America-Oceania	Spike: North America-Oceania	Spike: South America-North America
(U92, NA6723)	(U3, O5)	(NA992, O5)	(NA6751, O398)	(NA3313, SA1)
(U93, NA6775)	(U26, O43)	(NA3873, O28)	(NA6962, O400)	(NA4550, SA5)
(U94, NA6862)	(U30, O58)	(NA4024, O36)	(NA7060, O401)	(NA4720, SA7)
(U98, NA7057)	(U52, O201)	(NA4243, O43)	(NA7090, O402)	(NA4989, SA11)
(U99, NA7090)	(U63, O377)	(NA4508, O58)	(NA7230, O404)	(NA5595, SA13)
(U100, NA7129)	(U80, O390)	(NA4756, O65)	(NA7355, O415)	(NA5687, SA18)
(U103, NA7199)	(U99, O402)	(NA4861, O83)	(NA7402, O419)	(NA6101, SA19)
(U104, NA7312)	(U118, O1032)	(NA5011, O105)	(NA7510, O422)	(NA6146, SA20)
(U106, NA7431)	(U181, O1104)	(NA5041, O114)	(NA7811, O625)	(NA6161, SA21)
(U107, NA7557)		(NA5188, O148)	(NA7832, O631)	(NA6185, SA22)
(U111, NA7679)	Spike: Europe-South America	(NA5194, O201)	(NA7845, O633)	(NA6299, SA25)
(U112, NA7884)	(U9, SA1)	(NA5200, O225)	(NA7901, O645)	(NA6373, SA27)
(U113, NA7914)	(U41, SA11)	(NA5205, O238)	(NA8514, O751)	(NA6395, SA28)
(U114, NA9075)	(U63, SA13)	(NA5372, O368)	(NA8646, O770)	(NA6396, SA29)
(U116, NA9180)	(U80, SA32)	(NA5538, O370)	(NA8703, O798)	(NA6406, SA30)
(U117, NA9189)	(U84, SA35)	(NA5579, O374)	(NA8787, O850)	(NA6418, SA31)
(U119, NA9264)	(U99, SA42)	(NA5595, O377)	(NA8817, O886)	(NA6429, SA32)
(U121, NA9283)	(U124, SA63)	(NA5819, O387)	(NA8824, O889)	(NA6515, SA33)
(U122, NA9284)	(U181, SA71)	(NA6334, O388)	(NA9091, O1017)	(NA6622, SA35)
(U123, NA9330)		(NA6395, O389)	(NA9333, O1035)	(NA6696, SA38)
(U126, NA9458)		(NA6429, O390)	(NA9350, O1037)	(NA7015, SA41)
(U131, NA10312)		(NA6577, O391)	(NA9639, O1059)	(NA7090, SA42)
(U137, NA10457)		(NA6578, O392)	(NA9792, O1076)	(NA7430, SA43)
(U141, NA10669)		(NA6620, O395)	(NA9891, O1079)	(NA7477, SA44)
(U144, NA10811)			(NA13635, O1104)	(NA7521, SA45)
(U146, NA10987)				(NA7892, SA56)

(*continued on next page*)

Table 11 (*continued*)

Spike: Europe-North America	Spike: Europe-Oceania	Spike: North America-Oceania	Spike: North America-Oceania	Spike: South America-North America
(U148, NA11013)				(NA9324, SA61)
(U151, NA11225)				(NA9910, SA66)
(U153, NA11367)				(NA10342, SA68)
(U154, NA11456)				(NA13390, SA70)
(U155, NA11466)				(NA13635, SA71)
(U158, NA13110)				
(U160, NA13253)				
(U175, NA13414)				
(U177, NA13551)				
(U179, NA13626)				
(U181, NA13635)				
(U187, NA14000)				

Table 12
List of spike proteins from Asia, which were found to be identical with spike proteins from other five continents.

Spike proteins (Asia) which were found to be identical with spike proteins from other five continents								
A1	A71	A115	A171	A207	A239	A280	A344	A388
A8	A76	A121	A173	A210	A244	A282	A345	A391
A12	A77	A122	A174	A211	A245	A283	A348	A394
A14	A78	A126	A175	A212	A247	A284	A351	A395
A15	A85	A127	A177	A213	A249	A286	A354	A396
A19	A89	A128	A178	A214	A253	A291	A356	A399
A23	A90	A133	A180	A215	A254	A292	A357	A401
A26	A91	A134	A181	A216	A255	A293	A358	A404
A28	A93	A135	A182	A217	A257	A304	A359	A405
A30	A95	A138	A183	A218	A258	A305	A360	A408
A31	A96	A140	A191	A219	A264	A322	A367	A413
A32	A97	A142	A193	A221	A265	A323	A373	A418
A33	A100	A148	A195	A222	A267	A324	A375	A419
A34	A101	A158	A196	A223	A273	A325	A378	A430
A36	A102	A159	A198	A224	A274	A333	A379	A431
A43	A103	A161	A199	A230	A275	A335	A380	
A44	A105	A163	A200	A233	A276	A340	A381	
A58	A109	A164	A201	A234	A277	A341	A383	
A67	A111	A166	A205	A235	A278	A342	A386	
A69	A114	A170	A206	A238	A279	A343	A387	

Table 13
List of spike proteins from Africa, which were found to be identical with spike proteins from other five continents.

Spike proteins (Africa) which were found to be identical with spike proteins from other five continents										
AF1	AF34	AF58	AF79	AF101	AF117	AF128	AF145	AF156	AF227	AF263
AF2	AF38	AF64	AF82	AF103	AF118	AF130	AF146	AF165	AF229	AF268
AF3	AF46	AF69	AF83	AF104	AF119	AF131	AF147	AF168	AF230	AF271
AF8	AF47	AF71	AF88	AF105	AF120	AF133	AF149	AF179	AF231	AF278
AF9	AF48	AF72	AF90	AF108	AF121	AF134	AF151	AF195	AF247	AF283
AF19	AF50	AF73	AF92	AF114	AF123	AF137	AF152	AF196	AF248	
AF31	AF51	AF76	AF99	AF115	AF125	AF138	AF154	AF223	AF254	

Table 14
List of spike proteins from Europe, which were found to be identical with spike proteins from other five continents.

Spike proteins (Europe) which were found to be identical with spike proteins from other five continents								
U2	U18	U41	U63	U85	U103	U117	U137	U158
U3	U22	U42	U66	U88	U104	U118	U141	U160
U4	U23	U45	U72	U90	U106	U119	U144	U175
U7	U26	U47	U76	U92	U107	U121	U146	U177
U8	U28	U52	U78	U93	U111	U122	U148	U179
U9	U30	U53	U79	U94	U112	U123	U151	U181
U10	U34	U54	U80	U98	U113	U124	U153	U187
U11	U36	U55	U82	U99	U114	U126	U154	
U17	U38	U57	U84	U100	U116	U131	U155	

Table 15
List of spike proteins from North America, which were found to be identical with spike proteins from other five continents.

Spike proteins (North America) which were found to be identical with spike proteins from other five continents										
NA7	NA3911	NA4837	NA5595	NA6161	NA6510	NA6810	NA7300	NA8703	NA9792	NA13390
NA231	NA3986	NA4861	NA5606	NA6178	NA6515	NA6816	NA7312	NA8787	NA9834	NA13404
NA377	NA3988	NA4897	NA5627	NA6185	NA6527	NA6848	NA7355	NA8817	NA9891	NA13414
NA389	NA4024	NA4989	NA5644	NA6193	NA6540	NA6857	NA7375	NA8824	NA9910	NA13438
NA390	NA4028	NA5001	NA5645	NA6240	NA6550	NA6862	NA7402	NA9075	NA10257	NA13444
NA402	NA4051	NA5011	NA5666	NA6244	NA6553	NA6903	NA7430	NA9091	NA10276	NA13465
NA902	NA4061	NA5022	NA5687	NA6258	NA6566	NA6916	NA7431	NA9180	NA10312	NA13478
NA928	NA4117	NA5041	NA5693	NA6276	NA6577	NA6936	NA7453	NA9189	NA10342	NA13551
NA992	NA4169	NA5083	NA5722	NA6293	NA6578	NA6944	NA7477	NA9264	NA10442	NA13554
NA1104	NA4243	NA5105	NA5744	NA6299	NA6602	NA6949	NA7510	NA9265	NA10457	NA13578
NA1131	NA4270	NA5137	NA5818	NA6305	NA6616	NA6953	NA7521	NA9283	NA10669	NA13614
NA1221	NA4296	NA5149	NA5819	NA6324	NA6620	NA6962	NA7553	NA9284	NA10811	NA13626
NA1298	NA4375	NA5151	NA5829	NA6334	NA6622	NA6969	NA7557	NA9324	NA10943	NA13635
NA1348	NA4394	NA5167	NA5830	NA6373	NA6628	NA7000	NA7576	NA9330	NA10987	NA13668
NA1445	NA4436	NA5182	NA5837	NA6388	NA6630	NA7015	NA7644	NA9333	NA11013	NA13704
NA2065	NA4448	NA5188	NA5874	NA6390	NA6659	NA7025	NA7679	NA9350	NA11135	NA13798
NA2680	NA4508	NA5194	NA5927	NA6395	NA6661	NA7056	NA7811	NA9425	NA11225	NA13841
NA3228	NA4537	NA5200	NA5977	NA6396	NA6683	NA7057	NA7832	NA9455	NA11305	NA13870
NA3313	NA4541	NA5202	NA5992	NA6399	NA6687	NA7060	NA7845	NA9458	NA11367	NA13913
NA3387	NA4550	NA5205	NA6060	NA6406	NA6696	NA7090	NA7884	NA9568	NA11456	NA13948
NA3438	NA4559	NA5282	NA6067	NA6418	NA6704	NA7129	NA7892	NA9592	NA11466	NA14000
NA3477	NA4620	NA5372	NA6071	NA6421	NA6707	NA7198	NA7901	NA9597	NA11560	NA14015
NA3583	NA4637	NA5471	NA6080	NA6424	NA6713	NA7199	NA7914	NA9612	NA11576	NA14026
NA3658	NA4658	NA5485	NA6096	NA6429	NA6723	NA7224	NA8509	NA9639	NA11874	
NA3752	NA4715	NA5490	NA6101	NA6445	NA6751	NA7227	NA8514	NA9663	NA13110	
NA3768	NA4720	NA5516	NA6142	NA6450	NA6756	NA7230	NA8519	NA9674	NA13253	
NA3797	NA4756	NA5538	NA6146	NA6465	NA6775	NA7249	NA8565	NA9724	NA13280	
NA3873	NA4780	NA5574	NA6148	NA6492	NA6780	NA7286	NA8570	NA9763	NA13307	
NA3895	NA4832	NA5579	NA6155	NA6499	NA6794	NA7299	NA8646	NA9776	NA13362	

Table 16
List of spike proteins from Oceania, which were found to be identical with spike proteins from other five continents.

Spike proteins (Oceania) which were found to be identical with spike proteins from other five continents						
O3	O105	O373	O392	O419	O770	O1037
O5	O114	O374	O395	O422	O798	O1059
O28	O148	O377	O398	O504	O850	O1076
O36	O201	O387	O400	O625	O886	O1079
O43	O225	O388	O401	O631	O889	O1104
O58	O238	O389	O402	O633	O1017	
O65	O368	O390	O404	O645	O1032	
O83	O370	O391	O415	O751	O1035	

Table 17
List of spike proteins from South America, which were found to be identical with spike proteins from other five continents.

Spike proteins (Oceania) which were found to be identical with spike proteins from other five continents						
SA1	SA13	SA22	SA29	SA35	SA44	SA66
SA4	SA18	SA25	SA30	SA38	SA45	SA68
SA5	SA19	SA26	SA31	SA41	SA56	SA70
SA7	SA20	SA27	SA32	SA42	SA61	SA71
SA11	SA21	SA28	SA33	SA43	SA63	

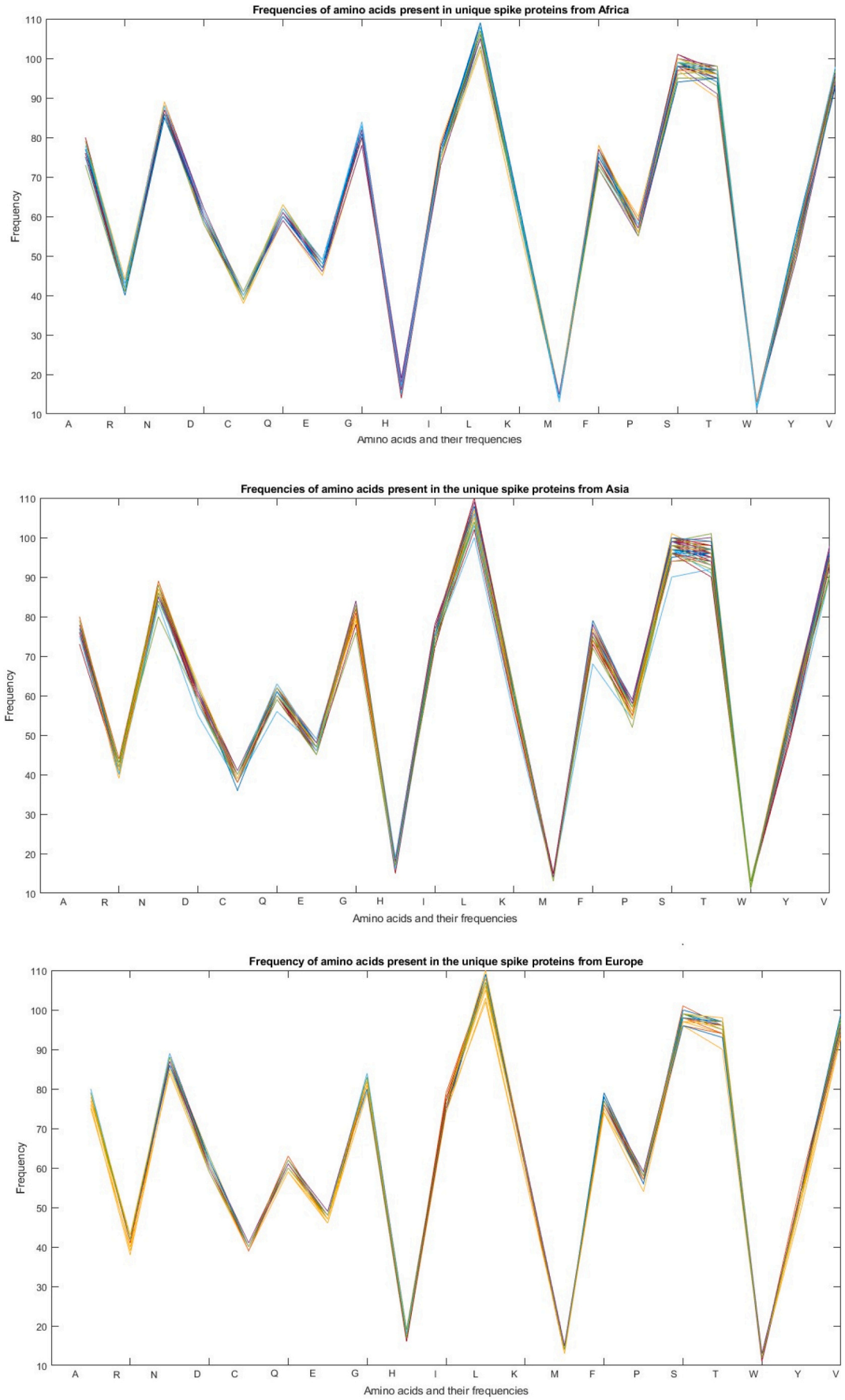

Fig. 6. Frequencies of amino acids present in the unique S sequences.

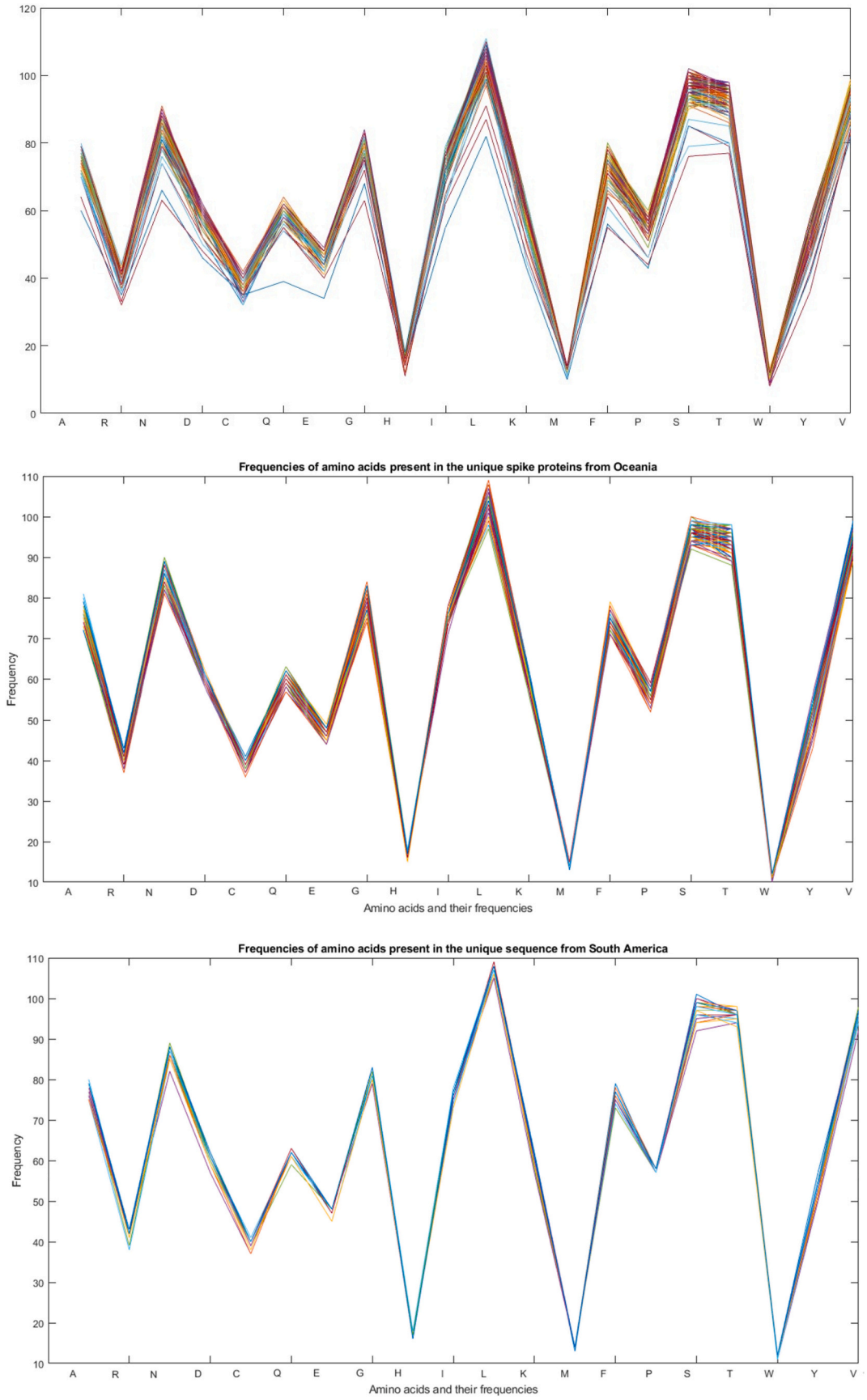

Fig. 7. Frequencies of amino acids present in the unique S sequences.

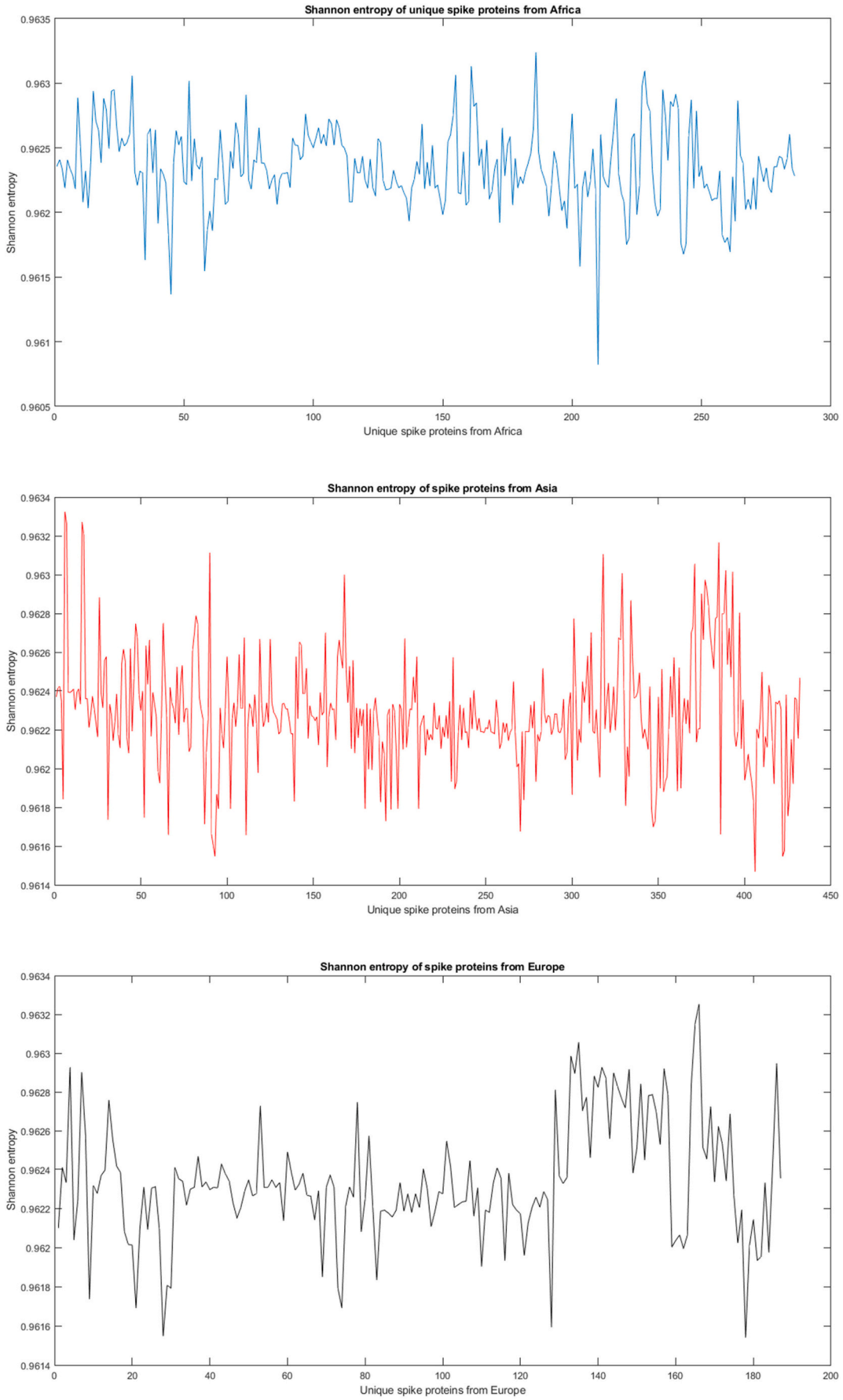

Fig. 8. SE of unique S proteins from different continents.

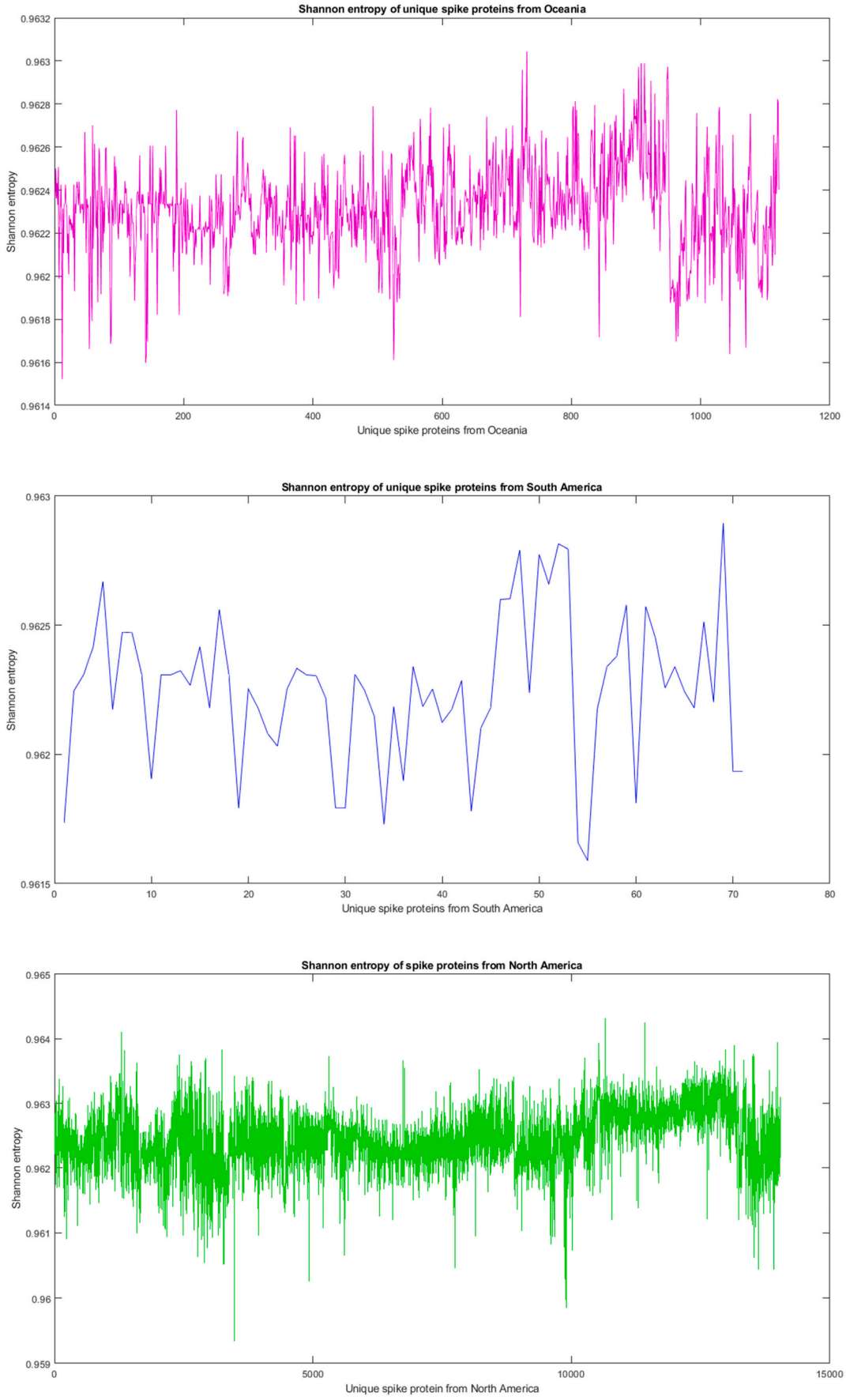

Fig. 9. SE of unique S proteins from different continents.

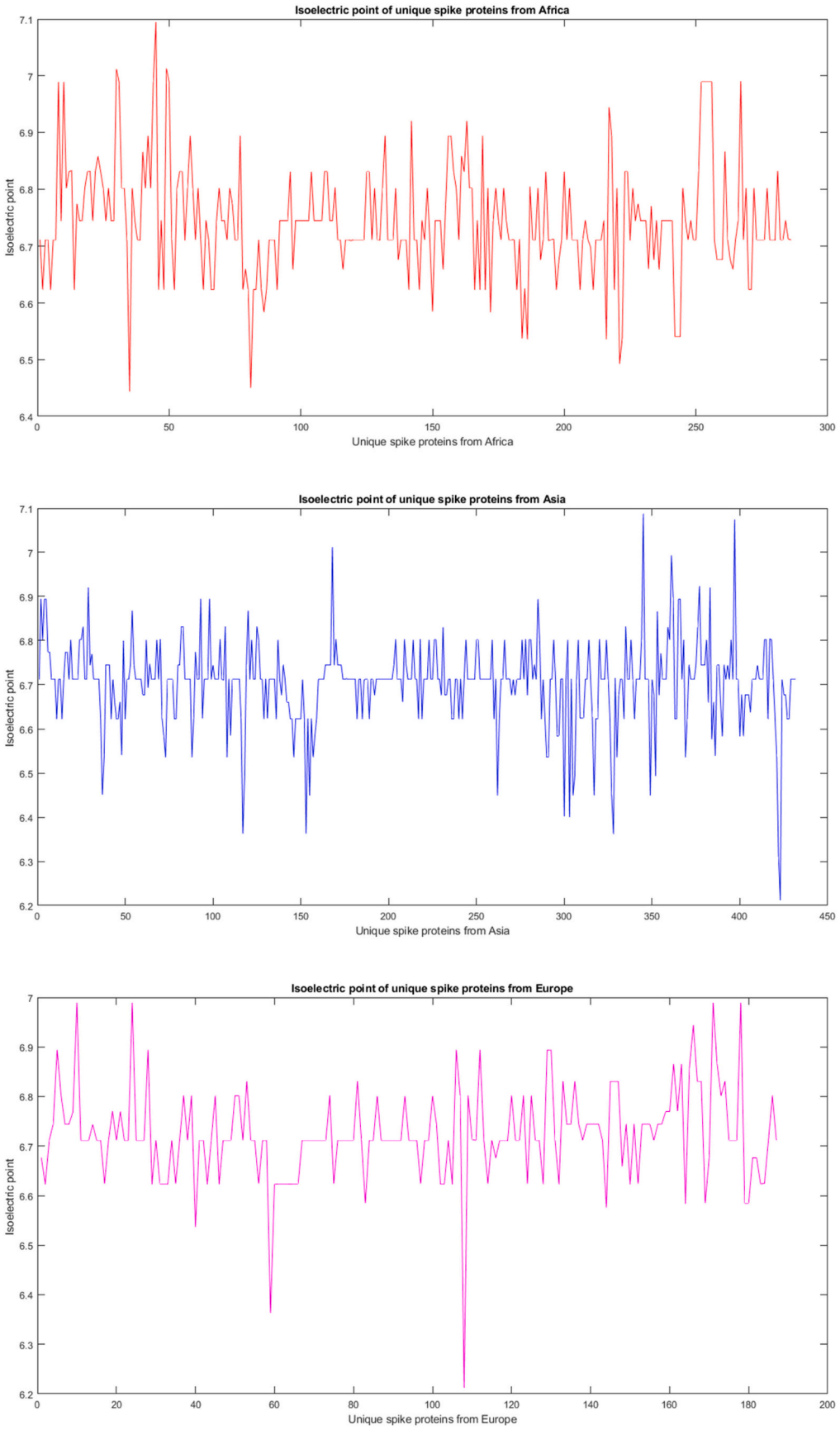

Fig. 10. Isoelectric point of unique S proteins from different continents.

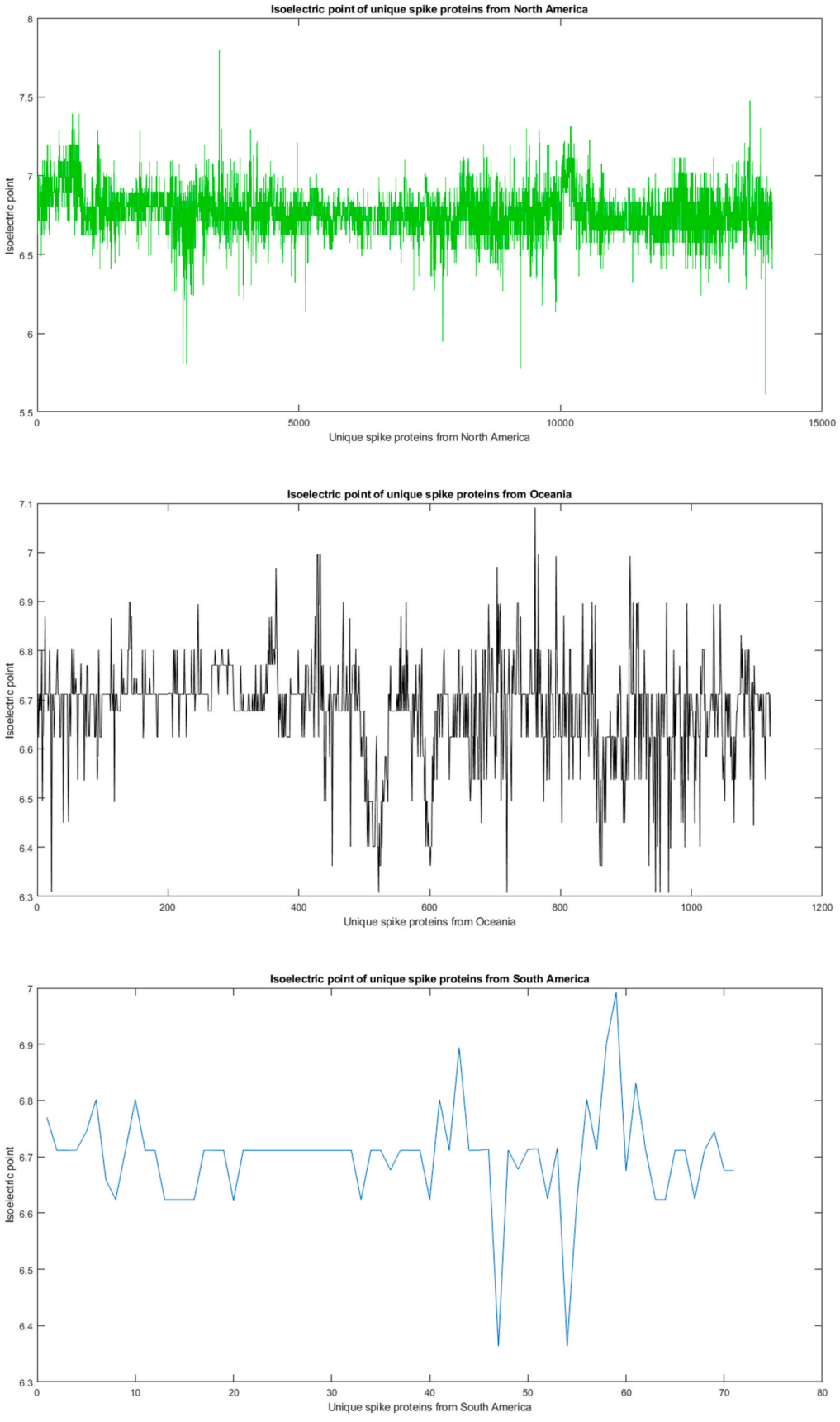

Fig. 11. Isoelectric point of unique S proteins from different continents.

References

[1] S.M. Lokman, M. Rasheduzzaman, A. Salauddin, R. Barua, A.Y. Tanzina, M. H. Rumi, M.I. Hossain, A.Mannan A. Z. Sid- diki M. M. Hasan, Exploring the genomic and proteomic variations of SARS-CoV-2 spike glycoprotein: a computational biology approach, Infect. Genet. Evol. 84 (2020), 104389.

[2] Á. Serrano-Aroca K. Takayama A. Tuñon-Molina M. Seyran S. S. Hassan P. P. Choudhury V. N. Uversky K. Lund- strom P. Adadi G. Palù et al., Carbon-based nanomaterials: promising antiviral agents to combat COVID-19 in the microbial resistant era, ACS Nano doi:10.1021/acsnano.1c00629. PMID: 33826850.

[3] S. Hassan, S. Ghosh, D. Attrish, P.P. Choudhury, A.A. Aljabali, B.D. Uhal, K. Lundstrom, N. Rezaei, V.N. Uversky, M. Seyran, et al., Possible transmission flow of SARS-CoV-2 based on ace2 features, Molecules 25 (24) (2020) 5906.

[4] M. Martı, A. Tuñon-Molina, F.L. Aachmann, Y. Muramoto, T. Noda, K. Takayama, Á. Serrano-Aroca, Protective face mask filter capable of inactivating SARS-CoV-2, and methicillin-resistant staphylococcus aureus and staphylococcus epidermidis, Polymers 13 (2) (2021) 207.

[5] S.S. Hassan, D. Attrish, S. Ghosh, P.P. Choudhury, V.N. Uversky, A.A. Aljabali, K. Lundstrom, B.D. Uhal, N. Rezaei, M. Seyran, et al., Notable sequence homology of the orf10 protein introspects the architecture of SARS-CoV-2, Int. J. Biol. Macromol. 181 (2021) 801–809.

[6] S.S. Hassan, A.A. Aljabali, P.K. Panda, S. Ghosh, D. Attrish, P.P. Choudhury, M. Seyran, D. Pizzol, P. Adadi, T.M.Abd El-Aziz, et al., A unique view of SARS-CoV-2 through the lens of ORF8 protein, Comput. Biol. Med. 133 (2021) 1–14, 104380.

[7] L. Zhang, C.B. Jackson, H. Mou, A. Ojha, H. Peng, B.D. Quinlan, E.S. Rangarajan, A. Pan, A. Vanderheiden, M.S. Suthar, et al., SARS-CoV-2 spike-protein d614g mutation increases virion spike density and infectivity, Nat. Commun. 11 (1) (2020) 1–9.

[8] L. Guruprasad, Human SARS-CoV-2 spike protein mutations, Proteins Struct. Funct. Bioinformatics 89 (5) (2021) 569–576.

[9] R. Henderson, R.J. Edwards, K. Mansouri, K. Janowska, V. Stalls, S.M. Gobeil, M. Kopp, D. Li, R. Parks, A.L. Hsu, et al., Controlling the SARS-CoV-2 spike glycoprotein conformation, Nat. Struct. Mol. Biol. 27 (10) (2020) 925–933.

[10] M. Seyran, K. Takayama, V.N. Uversky, K. Lundstrom, S.P. Sherchan, D. Attrish, N. Rezaei, A.A. Aljabali, S. Ghosh, G. Palù, et al., The structural basis of accelerated host cell entry by SARS-CoV-2, FEBS J. 288 (17) (2021) 5010–5020, https://doi.org/10.1111/febs.15651.

[11] I. Khatri, F.J. Staal, J.J. Van Dongen, Blocking of the high-affinity interaction-synapse between SARS-CoV-2 spike and human ACE2 proteins likely requires multiple high-affinity antibodies: an immune perspective, Front. Immunol. 11 (2020).

[12] G. Bauer, The potential significance of high avidity immunoglobulin g (IgG) for protective immunity towards SARS-CoV-2, Int. J. Infect. Dis. 106 (2021) 61–64.

[13] E.B. Hodcroft, D.B. Domman, D.J. Snyder, K. Oguntuyo, M.Van Diest, K. H. Densmore, K.C. Schwalm, J. Femling, J.L. Carroll, R.S. Scott, et al., Emergence in Late 2020 of Multiple Lineages of SARS-CoV-2 Spike Protein Variants Affecting Amino Acid Position 677, MedRxiv, 2021.

[14] Z. Ke, J. Oton, K. Qu, M. Cortese, V. Zila, L. McKeane, T. Nakane, J. Zivanov, C. J. Neufeldt, B. Cerikan, et al., Structures and distributions of SARS-CoV-2 spike proteins on intact virions, Nature 588 (7838) (2020) 498–502.

[15] O.A. MacLean, R.J. Orton, J.B. Singer, D.L. Robertson, No evidence for distinct types in the evolution of SARS-CoV-2, Virus Evol. 6 (1) (2020), veaa034.

[16] L. van Dorp, M. Acman, D. Richard, L.P. Shaw, C.E. Ford, L. Ormond, C.J. Owen, J. Pang, C.C. Tan, F.A. Boshier, et al., Emergence of genomic diversity and recurrent mutations in SARS-CoV-2, Infect. Genet. Evol. 83 (2020), 104351.

[17] J. Zhang, Y. Cai, T. Xiao, J. Lu, H. Peng, S.M. Sterling, R.M. Walsh, S. Rits-Volloch, H. Zhu, A.N. Woosley, et al., Structural impact on SARS-CoV-2 spike protein by D614G substitution, Science 372 (6541) (2021) 525–530.

[18] S.E. Park, Epidemiology, virology, and clinical features of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2; coronavirus disease-19), Clin. Exp. Pediatr. 63 (4) (2020) 119.

[19] E. Callaway, The coronavirus is mutating-does it matter? Nature 585 (7824) (2020) 174–177.

[20] B. Korber, W.M. Fischer, S. Gnanakaran, H. Yoon, J. Theiler, W. Abfalterer, N. Hengartner, E.E. Giorgi, B.Foley T. Bhat- tacharya, et al., Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus, Cell 182 (4) (2020) 812–827.

[21] E. Volz, V. Hill, J.T. McCrone, A. Price, D. Jorgensen, J.Southgate Á. O'Toole R. Johnson, et al., Evaluating the effects of SARS-CoV-2 spike mutation D614G on transmissibility and pathogenicity, Cell 184 (1) (2021) 64–75.

[22] T.C. Williams, W.A. Burgers, SARS-CoV-2 evolution and vaccines: cause for concern? Lancet Respir. Med. 9 (4) (2021) 333–335.

[23] H. Tegally, E. Wilkinson, M. Giovanetti, A. Iranzadeh, V. Fonseca, J. Giandhari, D. Doolabh, S. Pillay, N. Msomi, et al., Detection of a SARS-CoV-2 variant of concern in South Africa, Nature 592 (7854) (2021) 438–443.

[24] W. Shen, S. Le, Y. Li, F. Hu, Seqkit: a cross-platform and ultrafast toolkit for fasta/q file manipulation, PloS one 11 (10) (2016), e0163962.

[25] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, Mega x: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (6) (2018) 1547.

[26] R.C. Edgar, Muscle: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Res. 32 (5) (2004) 1792–1797.

[27] M.V. Han, C.M. Zmasek, Phyloxml: xml for evolutionary biology and comparative genomics, BMC Bioinformatics 10 (1) (2009) 1–6.

[28] D.J. Brooks, J.R. Fresco, A.M. Lesk, M. Singh, Evolution of amino acid frequencies in proteins over deep time: inferred order of introduction of amino acids into the genetic code, Mol. Biol. Evol. 19 (10) (2002) 1645–1655.

[29] V. Vacic, V.N. Uversky, A.K. Dunker, S. Lonardi, Composition profiler: a tool for discovery and visualization of amino acid composition differences, BMC Bioinformatics 8 (1) (2007) 1–7.

[30] M. Sickmeier, J.A. Hamilton, T. LeGall, V. Vacic, M.S. Cortese, A. Tantos, B. Szabo, P. Tompa, J. Chen, V.N. Uversky, et al., Disprot: the database of disordered proteins, Nucleic Acids Res. 35 (suppl 1) (2007) D786–D793.

[31] S.S. Hassan, D. Attrish, S. Ghosh, P.P. Choudhury, B. Roy, Pathogenetic perspective of missense mutations of orf3a protein of SARS-CoV-2, Virus Res. 300 (2021) 1–24, 198441.

[32] S.S. Hassan, P.P. Choudhury, B. Roy, S.S. Jana, Missense mutations in SARS-CoV-2 genomes from Indian patients, Genomics 112 (6) (2020) 4622–4627.

[33] B.J. Strait, T.G. Dewey, The shannon information entropy of protein sequences, Biophys. J. 71 (1) (1996) 148–155.

[34] P.G. Righetti, Determination of the isoelectric point of proteins by capillary isoelectric focusing, J. Chromatogr. A 1037 (1–2) (2004) 491–499.

[35] F.S. Stekhoven, M. Gorissen, G. Flik, The isoelectric point, a key to understanding a variety of biochemical problems: a minireview, Fish Physiol. Biochem. 34 (1) (2008) 1–8.

[36] G. Adair, The chemistry of the proteins and amino acids, Annu. Rev. Biochem. 6 (1) (1937) 163–192.

[37] P. Romero, Z. Obradovic, X. Li, E.C. Garner, C.J. Brown, A.K. Dunker, Sequence complexity of disordered protein, Proteins Struct. Funct. Bioinformatics 42 (1) (2001) 38–48.

[38] K. Peng, P. Radivojac, S. Vucetic, A.K. Dunker, Z. Obradovic, Length-dependent prediction of protein intrinsic disorder, BMC Bioinformatics 7 (1) (2006) 1–17.

[39] K. Peng, S. Vucetic, P. Radivojac, C.J. Brown, A.K. Dunker, Z. Obradovic, Optimizing long intrinsic disorder predictors with protein evolutionary information, J. Bioinforma. Comput. Biol. 3 (01) (2005) 35–60.

[40] B. Xue, R.L. Dunbrack, R.W. Williams, A.K. Dunker, V.N. Uversky, PONDR-FIT: a meta-predictor of intrinsically disordered amino acids, Biochim. Biophys. Acta (BBA)-Proteins Proteomics 1804 (4) (2010) 996–1010.

[41] G.Erdős B. Mészáros Z. Dosztányi, IUPred2a: context-dependent prediction of protein disorder as a function of redox state and protein binding, Nucleic Acids Res. 46 (W1) (2018) W329–W337.

[42] M. Necci, D. Piovesan, S.C. Tosatto, Critical assessment of protein intrinsic disorder prediction, Nat. Methods 18 (5) (2021) 472–481.

[43] A. Baum, B.O. Fulton, E. Wloga, R. Copin, K.E. Pascal, V. Russo, S. Giordano, K. Lanza, N. Negron, M. Ni, et al., Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies, Science 369 (6506) (2020) 1014–1018.

[44] Z. Liu, L.A. VanBlargan, L.-M. Bloyet, P.W. Rothlauf, R.E. Chen, S. Stumpf, H. Zhao, J.M. Errico, E.S. Theel, M.J. Liebeskind, et al., Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization, Cell Host Microbe 29 (3) (2021) 477–488.

[45] B. Dearlove, E. Lewitus, H. Bai, Y. Li, D.B. Reeves, M.G. Joyce, P.T. Scott, M. F. Amare, S. Vasan, N.L. Michael, et al., A SARS-CoV-2 vaccine candidate would likely match all currently circulating variants, Proc. Natl. Acad. Sci. 117 (38) (2020) 23652–23662.

[46] L.F. Buss, C.A. Prete, C.M. Abrahim, A. Mendrone, T. Salomon, C. de Almeida-Neto, M.C.Belotti R. F. Fran¸ca M. P. Carvalho, et al., Three-quarters attack rate of SARS-CoV-2 in the brazilian amazon during a largely unmitigated epidemic, Science 371 (6526) (2021) 288–292.

[47] C. Aschwanden, Five reasons why covid herd immunity is probably impossible, Nature 591 (7851) (2021) 520–522.

[48] R.K. Gupta, Will SARS-CoV-2 variants of concern affect the promise of vaccines? Nat. Rev. Immunol. (2021) 1–2.

[49] E.M. Redwan, COVID-19 pandemic and vaccination build herd immunity, Eur. Rev. Med. Pharmacol. Sci. 25 (2) (2021) 577–579.

[50] G. Bauer, The variability of the serological response to SARS-corona virus-2: potential resolution of ambiguity through determination of avidity (functional affinity), J. Med. Virol. 93 (1) (2021) 311–322.

[51] K. Hedman, M. Lappalainen, L.Hedman M. Soderlund, Avidity of IgG in serodiagnosis of infectious diseases, reviews in medical, Microbiology 4 (3) (1993) 123–129.

[52] A.K. Junker, P. Tilley, Varicella-zoster virus antibody avidity and IgG-subclass patterns in children with recurrent chickenpox, J. Med. Virol. 43 (2) (1994) 119–124.

[53] S.B. Boppana, W.J. Britt, Antiviral antibody responses and intrauterine transmission after primary maternal cytomegalovirus infection, J. Infect. Dis. 171 (5) (1995) 1115–1121.

[54] T. Lazzarotto, S. Varani, P. Spezzacatena, L. Gabrielli, P. Pradelli, B. Guerra, M. P. Landini, Maternal IgG avidity and igm detected by blot as diagnostic tools to identify pregnant women at risk of transmitting cytomegalovirus, Viral Immunol. 13 (1) (2000) 137–141.

[55] S. Seo, Y. Cho, J. Park, Serologic screening of pregnant korean women for primary human cytomegalovirus infection using IgG avidity test, Korean J. Lab. Med. 29 (6) (2009) 557–562.

[56] M. Kaneko, M. Ohhashi, T. Minematsu, J. Muraoka, K. Kusumoto, H. Sameshima, Maternal immunoglobulin G avidity as a diagnostic tool to identify pregnant women at risk of congenital cytomegalovirus infection, J. Infect. Chemother. 23 (3) (2017) 173–176.

[57] A. Puschnik, L. Lau, E.A. Cromwell, A. Balmaseda, S. Zompi, E. Harris, Correlation between dengue-specific neutralizing antibodies and serum avidity in primary and secondary Dengue virus 3 natural infections in humans, PLoS Negl. Trop. Dis. 7 (6) (2013), e2274.

[58] M.F. Delgado, S. Coviello, A.C. Monsalvo, G.A. Melendi, J.Z. Hernandez, J. P. Batalle, L. Diaz, A. Trento, H.-Y. Chang, W. Mitzner, et al., Lack of antibody affinity maturation due to poor toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease, Nat. Med. 15 (1) (2009) 34–41.
[59] L. Lai, P.A.Kozlowski D. Vodr̈os D. C. Montefiori, et al., GM-CSF DNA: an adjuvant for higher avidity IgG, rectal IgA, and increased protection against the acute phase of a SHIV-89.6P challenge by a DNA/MVA immunodeficiency virus vaccine, Virology 369 (1) (2007) 153–167.
[60] G. Bauer, F. Struck, P. Schreiner, E. Staschik, E. Soutschek, M. Motz, The Serological Response to SARS Corona Virus-2 Is Characterized by Frequent Incomplete Maturation of Functional Affinity (Avidity), 2020.
[61] A.W. Edridge, J. Kaczorowska, A.C. Hoste, M. Bakker, M. Klein, K. Loens, M. F. Jebbink, A. Matser, C.M. Kinsella, P. Rueda, et al., Seasonal coronavirus protective immunity is short-lasting, Nat. Med. 26 (11) (2020) 1691–1693.
[62] M. Galanti, J. Shaman, Direct observation of repeated infections with endemic coronaviruses, J. Infect. Dis. 223 (3) (2021) 409–415.
[63] F. Struck, P. Schreiner, E. Staschik, K. Wochinz-Richter, S. Schulz, E. Soutschek, M. Motz, G. Bauer, Vaccination versus infection with SARS-CoV-2: establishment of a high avidity igg response versus incomplete avidity maturation, J. Med. Virol. (2021).
[64] WHO-COVID-19-Dashboard, COVID live update. https://covid19.who.int/table. (Accessed 12 September 2021).
[65] S.L. Wu, A.N. Mertens, Y.S. Crider, A. Nguyen, N.N. Pokpongkiat, S. Djajadi, A. Seth, M.S. Hsiang, J.M. Colford, A. Reingold, et al., Substantial underestimation of SARS-CoV-2 infection in the United States, Nat. Commun. 11 (1) (2020) 1–10.
[66] M. Mohanan, A. Malani, K. Krishnan, A. Acharya, Prevalence of SARS-CoV-2 in Karnataka, India, Jama 325 (10) (2021) 1001–1003.
[67] R. Asselta, E.M. Paraboschi, A. Mantovani, S. Duga, Ace2 and tmprss2 variants and expression as candidates to sex and country differences in COVID-19 severity in Italy, Aging (Albany NY) 12 (11) (2020) 10087.
[68] A. Srivastava, A. Bandopadhyay, D. Das, R.K. Pandey, V. Singh, N. Khanam, N. Srivastava, P.P. Singh, P.K. Dubey, A. Pathak, et al., Genetic association of ACE2 RS285666 polymorphism with COVID-19 spatial distribution in India, Front. Genet. 11 (2020) 1163.
[69] R.Posadas-Śanchez G. Vargas-Alarcón J. Rańırez-Bello, Variability in genes related to SARS-CoV-2 entry into host cells (ACE2, TMPRSS2, TMPRSS11A, ELANE, and CTSL) and its potential use in association studies, Life Sci. 260 (2020), 118313.