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Origin and main features of gryphon, cerberus, kraken, orthrus, bythos, hyperion and arctur, the current SARS-CoV-2 variants

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Abstract
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the most serious pandemics affecting the world in recent years. It was first isolated in China in 2019 and then spread and identified worldwide. To date, due to the numerous mutations accumulated on the viral genome, hundreds of virus variants have been isolated. As a general trend, the SARS-CoV-2 variants identified so far have increased their diffusion and reduced their danger and mortality. About 15 million viral sequences were carried out to control the spread of the virus and its variability. However, due to the reduced danger, from December 2022 to May 2023 a reduction was observed in the viral sequences collected and entered in the GISAID database (Global Initiative on Sharing Avian Influenza Data; https://gisaid.org) from 99950 to 25415 sequences, respectively. At the beginning of 2022, a new group of variants was isolated, referred to as omicron, which in a few months replaced all the variants present up to then. Here, we have examined the latest variants BQ.1, BQ.1.18 (CH.1.1), XBF, XBB, XBB.1.5, XBB.1.9 and XBB.1.16, indicating their origin and their derivation from the BA.2.12.1 and BA.5 variants, respectively, by analyzing the new mutations exhibiting these variants. Based on these studies, we proposed a new phylogenetic tree of omicron variants.

Keywords: COVID-19; SARS-CoV-2; SARS-CoV-2 variants of concern; phylogenetic analysis; variants diffusion.

1. Introduction

Coronavirus disease 2019 (COVID-19), the pandemic due to the SARS-CoV-2 infection, reported for the first time in the city of Wuhan (China) in December 2019 [1], has posed a significant threat to public health. Over the past three years, we have been cyclically observing new waves of infections, due to the evolution of SARS-CoV-2 in new variants, capable of evading surveillance systems as well as eluding developed vaccines, impacting strongly people's health and lives.

At the end of May 2023, data from the World Health Organization reported over 750 million confirmed cases and over 6.9 million deaths by COVID-19 (https://www.who.int, document: 20230525_weekly_epi_update_144.pdf).

More information about the origin of the virus and its mechanism of infection and replication has been clarified in the last three years. We currently know that SARS-CoV-2 is a coronavirus, that belongs to the *Coronaviridae* group [2]; membrane spike glycoprotein (S), the most abundant viral protein, is involved in the mechanism of viral infection [1]; and it has been used as an antigen for vaccine production [3], as well as its mutations have been used to classify variants of the SARS-CoV-2 [4].

The effect of mutations on the spike protein resulted in an alteration of the mechanisms of viral infection and propagation, and consequently in milder symptoms and/or increased viral spreading [5]. This led to speculation that the SARS-CoV-2 infection was turning from pandemic to endemic [6]. To date, hundreds of mutations on the viral genome have been sequenced. Many of them have been supplanted or eliminated during the viral evolution while, only a small number gave rise to new SARS-CoV-2 variants, and from these last ones, alpha, beta, gamma, delta, and omicron variants have been reported as Variant of Concern (VOC) [4].

The current globally circulating dominant variants are derived from omicron, accounting for nearly all sequences reported into the GISAID database (Global Initiative on Sharing Avian Influenza Data; https://gisaid.org). It has been observed that the new variants, in a few weeks from their isolation and sequencing, showed an increasing diffusion compared to the others. Further, since these variants could originate new waves of contagion all over the world, they have been classified as Variant of Interest (VOI) and their monitoring is critical. Since the last isolated variants derive from omicrons, which are VOCs, they have been considered by WHO for their potential diffusion and replacement of previous variants, and for these reasons they have been classified as Variant Under Monitoring (VUM) (https://www.who.int/news/item/16-03-2023-statement-on-the-update-of-who-s-working-definitions-and-tracking-system-for-sars-cov-2-variants- by -concern-and-variants-of-interest). The sequences submitted to GISAID from the beginning of the pandemic are 15,670,000.

1.1. omicron variants evolution

In November 2021, about two years after the first isolation of SARS-CoV-2, the variant named omicron was isolated [6]. To date, this variant has accumulated several mutations, generating changes in the main features of the virus and resulting in eight different new omicron variants. The origin of the omicron lineage may be derived from a common early ancestor with the delta variant [7]. Two mutations, T478K and D614G, were identified on the spike protein of both delta and all the omicron variants, suggesting their common origin. However, we cannot exclude that the omicron' origin could be derived by events of genomic recombination in two VOCs, infecting patients, simultaneously.

In the omicron variants the mutations are not only on the spike protein but also throughout the whole genome, in fact, the BA.4 and BA.5 omicron variants have the same 31 mutations on the spike protein, but they differ for another 20 mutations on the genome [7]. In particular, BA.4 exhibits specific mutations in N protein, nonstructural protein 1 (NSP1) and ORF7b, while BA.5 shows specific mutations in ORF6 and membrane M protein [8]. The first isolated omicron variant BA.1 (omicron 1) displayed 34 mutations on the spike protein [7]. The second isolated omicron variant BA.2 (omicron 2), originated from a common ancestor with BA.1, as it shows only seventeen common mutations with the BA.1 spike protein, while in total BA.2 has 28 mutations on spike protein, indicating a separate evolution between these two variants [7]. The third isolated omicron BA.2.12.1, known as omicron 3, evolved from BA.2. The variants BA.4, BA.5 and BA.2.75, known as omicron 4, omicron 5 and centaurus, respectively, originated separately from omicron BA.2.12.1 [7].

1.2. the Omicron variants: symptoms and their main features

SARS-CoV-2, isolated in 2019, was characterized by a high mortality and spreading rate compared to other viral infections that appeared in the last decade [9]. SARS-CoV-2 infection begins after an incubation time ranging from 2 to 4 days. It is characterized by dry cough and low-grade fever within 3 days from infection (38–39°C or 100,5–102,1°F). Mostly, the infection remains mild to moderate, and the symptoms resolve within one week [10]. Schematically, the timeline of COVID-19 disease could be divided into four stages: stage 0), a pre-illness stage; stage 1), the phase of viral infection and amplification, characterized by dry cough, fever and fatigue; stage 2), viral expansion to other organs and the initiation of immune response, characterized by severe breathing difficulties; stage 3), inflammation by a cytokine storm in response to viral infection, which led to pulmonary failure, multiple organ failure and death in severe cases [11].

In the first phase of the pandemic in early 2020, the infection was accompanied by other symptoms associated with an increased risk of developing more severe COVID-19 disease, affecting the respiratory, musculoskeletal, gastrointestinal, skin, nervous and cardiovascular systems [10]. Among all the SARS-CoV-2 variants, the last one isolated and called omicron, exhibited different characteristics than the previous ones, showing milder symptoms despite being highly infectious and transmissible [12]. Five major omicron lineages were isolated from December 2021 to September 2022, with BA.2 sublineage being the dominant strain over all the others [13]. From BA.2 evolved the BA.2.12.1 sublineage, denoted as omicron BA.3, from which derive BA.4, BA.5 and BA.2.75 (unofficially referred to as centaurus) [7].

BA.2.75 has been isolated in May 2022 in many countries [14]. It is characterized by having thirty-four mutations on the spike protein, seven of which are unique and specific to this variant, and are K147E, W152R, F157L, I210V, G257S, G446N and N460K [7, 15-16]. This could explain the immune escape capacity and resistance to antibody therapies shown by this variant compared to the previous ones [16]. The symptoms, associated to BA.2.75, are those of a seasonal flu: fever, cough, diarrhea and tiredness [16].

1.3. spike protein S

SARS-CoV-2 spike protein S is a glycoprotein of 1273 amino acids residues (Figure 1); it is a homotrimer localized on the virus surface in a high number of copies. The receptor binding domain (RBD; residues from 319 to 529) is responsible for the binding to the host cell by the recognition of the angiotensin converting enzyme 2 (ACE-2), it has been found that many mutations on the RBD domain originated new virus variants [6].

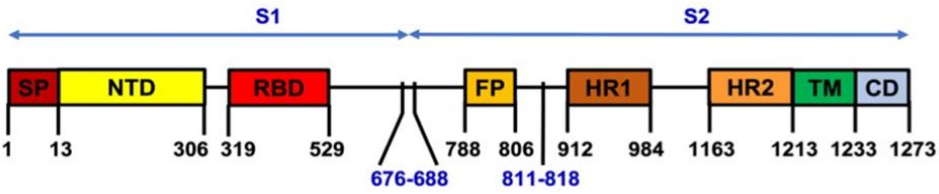

Fig. 1. Schematic representation of spike protein. The site 676-688 is recognize from furin and divided spike protein in two sub-domains S1 and S2; the site 811-818 is recognize from TMPRSS2 protease during the infection mechanism. SP is the signal peptide (residues 1-13); NTD is the N-Terminal domain (residues 14-306); RBD is the receptor-binding domain (residues 319-529). NTD and RBD are responsible for the binding to ACE-2 receptor; FP is the fusion peptide (residues 788-806); HR1 is the heptapeptide repeat sequence 1 (residues 912-984); HR2 is the heptapeptide repeat sequence 2 (residues 1163-1213); TM is the transmembrane domain (residues 1214-1233); CD is the cytoplasmatic domain (residues 1234-1273).

The mutations accumulated on the spike protein had a double effect: on the one hand they increased the binding affinity of the spike protein to the human ACE-2 receptor, leading to a greater spread of the virus; on the other, they can reduce the affinity against the neutralizing antibodies [17]. These mutations conferred an evolutionary advantage for the virus. This effect is evident for the omicron variants which accumulated 30 to 50 mutations compared to the initial version of the virus. In omicron BA.1, 15 mutations are in the RBD spike domain, which is also the target of neutralizing antibodies [17]. Some studies have indicated that omicron BA.1 is three to six times more infectious than the previous variants [18]. Studies have been conducted to measure the neutralizing capacity of vaccine-induced immunity against omicron variants, and in the case of omicron BA.1, a marked reduction in the neutralizing capacity of approximately 10-fold the vaccine efficacy has been demonstrated [17, 19].

The omicron variant BA.2, compared with BA.1, having additional mutations in the RBD spike domain (S371F, T376A, D405N, R408S), showed higher antibody evasion than BA.1 [17, 20]. It has been observed that some substitutions in the RBD domain of the omicron variants conferred a higher binding affinity to ACE2. Interestingly, some of them, notably S373P, S375F, T478K Q498R, and N501Y, are common to all the omicron variants, while others such as the Q493R substitution is present in BA.1, BA.2 and BA.2.12.1; S371L only in BA.1, and S371F in all the other omicron variants. [17, 21].

The omicron variants BA.4 and BA.5 have been shown to have other mutations in the spike protein. In particular, it has been observed that the L452R substitution, is associated with their higher transmissibility [22], and together with the other spike mutations it seems to be responsible for their ability to evade neutralizing antibodies more easily than the previous variants [23].

In terms of the basic reproduction number, R_0, which provides indications about the contagiousness and transmissibility of infectious pathogens, in general, for the BA.1 variant it was estimated to be respectively two- and about fourteen-folds higher than the delta variant and initial version [24], making the omicron variants the most transmissible SARS-CoV-2 variants [25].

2. Materials and Methods

2.1. SARS-Cov-2 diffusion analysis

The data related to the sequences number and variants diffusion were from WHO database, we consider the reports "COVID-19 Weekly Epidemiological Update" from December 2022 up to May 2023 (https://www.who.int, document: 20230125_Weekly_Epi_Update_127.pdf; 20230222_Weekly_Epi_Update_131.pdf; 20230330_Weekly_Epi_Update_136.pdf; 20230427_Weekly_Epi_Update_140.pdf; 20230525_Weekly_Epi_Update_144.pdf) and from GISAID (Global Initiative on Sharing Avian Influenza Data; https://gisaid.org).

2.2. sequence analysis

The omicron variants sequences of SARS-CoV-2 spike protein were from "expasy viralzone" web site (https://viralzone.expasy.org/9556). Successively, to generate the phylogenetic three of the omicron variants, multiple sequence alignment was performed by using Clustal Omega program (Multiple Sequence Alignment at https://www.ebi.ac.uk/Tools/msa/clustalo/).

3. Results

In recent months, the perception of the danger of SARS-CoV-2 has decreased. In many countries, controls and restrictions have been minimized and the number of genomic sequences of the virus carried out from December 2022 to May 2023 and reported into the GISAID database, decreased from about 100,000 to about 25,000 (Table 1). However, the virus has continued to be active and in recent months a number of new variants have been isolated, which according to their spreading have been kept under control by the World Health Organization (WHO). In particular, the new variants, indicated as BA.2.75 (centaurus), BA.5.2.1.7 (BF.7, a sublineage derived from BA.5), BQ.1(cerberus), BQ.1.18 (CH.1.1/orthrus), XBB (gryphon), XBB.1.5 (kraken), and XBB.1.16 (arctur), are rapidly spreading in several countries and are annotated as Variant Of Interest (VOI), while the last variants XBF (bythos) and XBB.1.9 (hyperion) are annotated as Variant Under Monitoring (VUM) (https://www.who.int).

The use of fantasy names for omicron variants, such as centaurus, cerberus, gryphon, kraken, bhytos, hyperion, orhtrus, and arctur, is misleading because these variants appear as new evolutionary lines completely different from those previously studied. Instead, in this case, they derive from omicron BA.5 or BA.2.12.1. Moreover, the new variants showed different features mainly due to the accumulation of mutations on the genome. In this study, a phylogenetic tree of these new variants was generated by multisequence alignment (see Materials and supplemental data). In particular, were used the spike sequences from the BA.1, BA.2, BA.2.12.1 (omicron 3), BA.4, BA.5, BA.2.75 (centaurus), XBB (gryphon) and BQ.1 (cerberus) variants, while the sequences from the latest BA.5.2.1.7 (BF.7), BQ.1.18 (CH.1.1/orthrus), XBB.1.5 (kraken), XBB.1.9 (hyperion), XBB.1.16 (arctur), and bythos (XBF) variants are not been considered, being sublineages of the other omicron variants.

3.1. variants spreading

During the last six months, we have followed the data on the spreading of the isolated omicron variants. As reported in Table 1, in December 2022 the most widespread were BA.5 and BQ.1 (Cerberus), respectively with 43 and 29 % of genomes sequenced, both in March 2023 were decreased to about 6 %. In December 2022, BA.4 was at 1.2 %, but then, it disappeared completely in two months. The BA.2.75 variant (centaurus) showed a peak in January 2023 (16 % of total sequences) and since March 2023 it is around 1 %.

Table 1: List of the most widespread SARS-CoV-2 variants from December 2022 to May 2023, as reported by WHO. The data related to the omicron variants spreading were from the sequences shared through GISAID (Global Initiative on Sharing Avian Influenza Data; https://gisaid.org) and WHO. The data are reported as percentage of the total number of sequences reported for each month.

month	Number of sequences**	**Variants**										
		BA.4	BA.5	BA.2.75	XBB	XBB.1.5	XBB.1.9	XBB.1.16	BQ.1	BQ.1.18	XBF	* Not assigned
December 2022	99950	1.2	43.1	9.8	4.3	-	-	-	29.4	-	-	12.2
January 2023	78505	0.4	40.0	16	9.9	-	-	-	28.1	-	-	5.4
February 2023	67081	<0.2	19.5	7.6	6.3	29	-	-	22.7	-	-	12.9
March 2023	54922	-	6.8	1.7	9.7	45	-	-	8.4	6.4 %	-	11.9
April 2023	35474	-	< 1	1.7	13	45.4	12	4.3	3.6	4 %	12	14.7
May 2023	25415	-	-	1.1	10.8	41.4	20.7	13.1	0.3	2.3 %	20.7	9.6

*Variants with different mutations derived from others omicron not reported in table.
**data from https://www.who.int, "weekly epidemiological update", see Materials.

The variant XBB, unofficially named gryphon, and its derivatives (XBB.1.5, XBB.1.9 and XBB.1.16), from December 2022 showed the greatest diffusion compared to the others, originating new waves of contagion worldwide; in particular, XBB remained around 10 %, while XBB.1.5 (kraken) from March to today is the most common variant, resulting in more than 40 % of the total sequences (Table 1). Instead, the last two variants XBB derived, XBB.1.9 and XBB.1.16 are increased up to 20 and 13 %, respectively in May 2023 (Table 1).

Finally, the last emerging variant XBF (bythos) was confirmed in April 2023 at 12 % and in May was increased at 20 % of total sequences (Table 1).

3.2.omicron variants evolution

The most impressive data about SARS-CoV-2 is the high number of mutations isolated that have modified its features. It has been previously demonstrated that some intermediate variants, during the evolution of SARS-CoV-2, accumulated new mutations without generating defined variants, as these were rapidly replaced by the new variants that had accumulated other mutations in the meantime [7]. Just to clarify this point, if we consider the BA.1 and BA.2 variants, they have not evolved from each other, but they have a common ancestor (intermediate variant, Figure 2) that shares twenty common mutations on the spike protein of both, BA.1 and BA.2, and which are: G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H and N969K.

BA.1 has eighteen other mutations, nine of which are not present on other omicron variants (A67V, DEL69-70, T95I, DEL142-145D, DEL211-212, G496S, T547K, N856K and L981F), while BA.2 lacks thirteen mutations present in BA.1 but displays four mutations not present on the BA.1 spike protein (T19I, DEL24-27S, G142D and V213G) [7].

Following the same reasoning, omicron BA.4 and BA.5 evolved from BA.2.12.1 (omicron 3). They exhibit the same mutations on the spike protein and differ of about twenty mutations on the rest of the genome [26]. Therefore, it has been postulated the presence of an intermediate variant between them, carrying only the mutations identified on the spike protein in BA.4 and BA.5 and not on the other viral genome regions, where other mutations occurred separately during the evolution of BA.4 and BA.5 [7].

It is evident that the new and unique mutations accumulated on spike protein are of interest to discriminate new variants and/or the intermediate among them. In Table 2, the new mutations on the spike protein of the last omicron variants are reported. These mutations are crucial in order to assign the new variants to the specific evolution sublineages, as reported in the phylogenetic tree (Figure 2).

3.3.BA.5.2.1.7 (BF.7) and BQ.1 (cerberus) variants

Sequence analysis of these variants, by means of alignment (Supplemental data), allowed to clearly highlight that the BQ.1/cerberus variant evolved from BA.5. Indeed, its spike protein has all the mutations present in BA.5 plus two new mutations: R346T and K444T, while BA.2.1.7 (BF.7) has the mutations present in BA.5 plus K444T and D1199N, indicating that both derived from BA.5 by divergent lineages; while in the case of the variant BA.5.2.1.7 (BF.7) the new spike mutation D1199N (Table 2) was found giving it new features.

From BQ.1 derived BQ.1.18 (CH.1.1/orthrus), which have other two new mutations: deletion Y144 and T376A (Table 2).

Table 2. Unique mutations and mutations present in a restricted number of subvariant identified in spike proteins from the omicron variants and subvariants BA.2.12.1, BA.2.75, XBB, XBB.1, XBB.1.5, XBB.1.9, XBB.1.16, XBF, BA.5.2.1.7, BQ.1 and BQ.1.18, to highlight their possible evolution.

variants	BA.2.12.1 (omicron 3)	BA.2.75 (centaurus)	XBB (gryphon)	XBB.1	XBB.1.5 (kraken)	XBB.1.9 (hyperion)	XBB.1.16 (arctur)	XBF (bythos)	BA.5.2.1.7 (BF.7)	BQ.1 (cerberus)	BQ.1.18 (CH.1.1 or orthrus)
mutations									S33F		
						V62I					
			V83A								
	Y144 del		Y144 del								Y144 del
			H146Q								
		K147E						K147E			
		W152R						W152R			
		F157L						F157L			
							E180V				
			Q183E	Q183E	Q183E	Q183E	Q183E				
		I201V						I201V			
				G252V	G252V	G252V	G252V				
						S256L					
								I257E			
		G275S									
	G339D	G339H	G339H	G339H	G339H	G339H	G339H	G339H			
			R346T	R346T	R346T	R346T	R346T	R346T	R346T	R346T	R346T
			L368I	L368I	L368I	L368I	L368I				
									S375A	S375A	
											T376A
									K444T	K444T	K444T
			V445P	V445P	V445P	V445P	V445P				
		G446S						G446S	G446S	G446S	G446S
	Y452L										
			N460K	N460K	N460K	N460K	N460K	N460K	N460K	N460K	N460K
					S478R	S478R	S478R			T478K	T478K
			F486S	F486S	F486P	F486P	F486P	F486P			
			F490S	F490S	F490S			F490S	F490S	F490S	F490S
	S704L										
									D1199N		

3.4.XBB lineage

Regarding the origin of the XBB variant, it has been reported that derived from a recombination between two BA.2 lineages: BJ.1 (BA.2.10.1, called also argus) and BA.2.75 [27]. The sequence alignment that we obtained and the related phylogenetic tree (Figure 2), confirmed that XBB evolved from the recombination of BJ.1 and BA.2.75.

As reported in Table 1, XBB was present starting from December 2022 and today it is still present in many countries. Due to its rapid spreading in many countries, it generated new variants, and one of them has been indicated as kraken, like the legendary sea monster, but it is a sub-lineage of XBB; in fact, it is correctly reported as XBB.1.5, and it has been isolated also the intermediate variant between XBB and XBB.1.5, which is XBB.1.

XBB.1 differs from XBB by the mutation G252V, whereas XBB.1.5 retains the mutation G252V but it changed the mutation F486S in F486P, compared to XBB and XBB.1 (Table 2). The substitution at position 486 has been indicated as critical for the immune response of these variants, leading to an increased transmissibility, as observed [28].

Since April 2023, other two new variants evolved from XBB: XBB.1.9 (hyperion) and XBB.1.16 (arctur). The genome of both variants was sequenced in 20 and 13 % of the total sequences, respectively, in May 2023. XBB.1.9 has been defined a Variant Under Monitoring (VUM) (https://github.com/cov-lineages/pango-designation/issues/1980) since its appearance; it shows only two new mutations: V62I and S256L. Instead, XBB.1.16 (arctur) has been classified as Variant Of Interest (VOI) (https://github.com/cov-lineages/pango-designation/issues/1723), and it is characterized by the new mutation E180V and by the change of T478R in T478K (Table 2). All the variants XBB1.5, 1.9 and 1.16 are sublineages of XBB.1 (Figure 2).

3.5.XBF variant (bythos)

Another new SARS-CoV-2 variant, annotated as XBF (bythos) has been isolated at the end of 2022. This new that caused an increase in infections in Australia and Sweden [29], and actually is a Variant Under Monitoring (VUM). The unofficial name bythos, referred to the mythological sea monster having human body, horse legs and fish tail, is not casual because this variant seems originated from the recombination of two or three variants, in particular it has been reported as a recombination between BA.5 and BA.2.75 or BQ variants [29].

3.6.omicron phylogenetic tree

Based on the spike protein sequence's alignment of these variants (see supplemental) we generated a new phylogenetic tree. As illustrated in Figure 2, it is evident that BA.1 and BA.2 evolved separately from a common intermediate, while omicron 3 (BA.2.12.1) derived from BA.2.

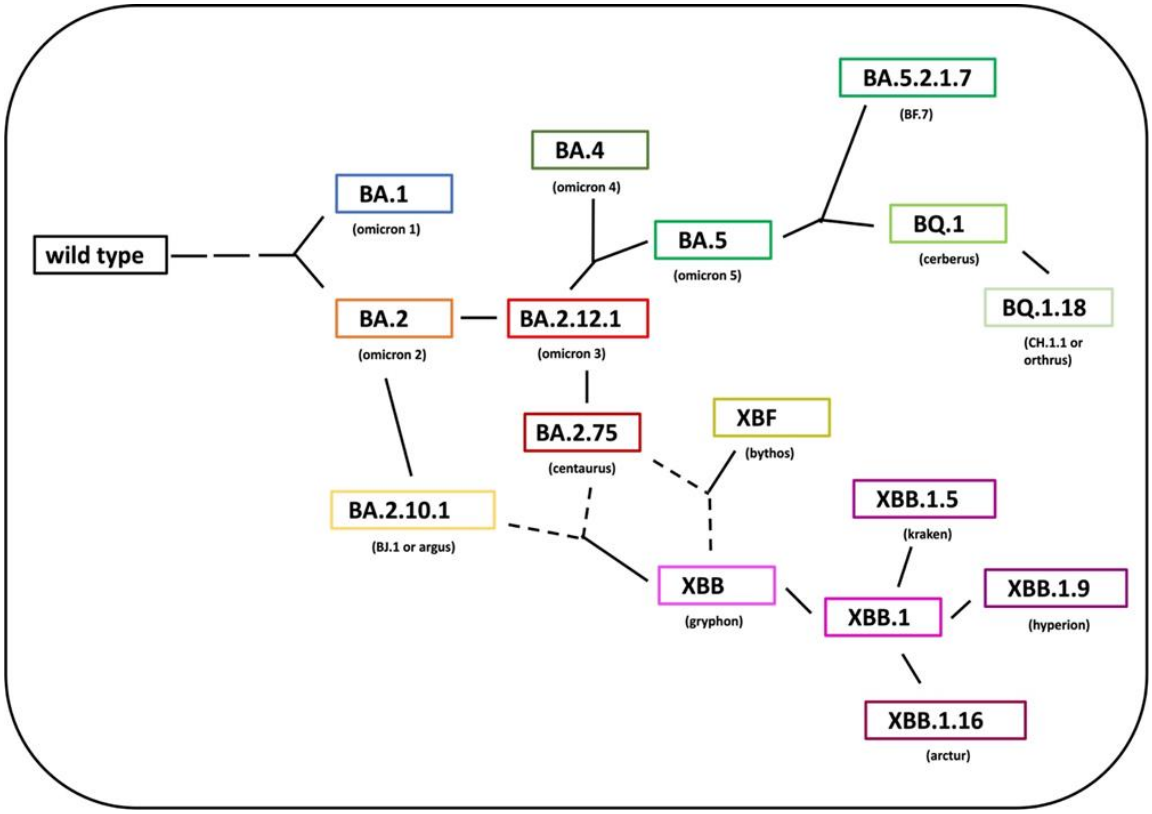

Fig. 2. Phylogenetic lineage of the omicron variants, based on their spike proteins sequence alignment. BA.1 and BA.2 evolved separately from a common intermediate; omicron 3 (BA.2.12.1) derived from BA.2. From omicron 3 derived two separate lineages, one is related to BA.5-BQ.1 variants, and the second is related to the BA.2.75-XBB variants. XBF is the results of a recombination between BA.2.75 and XBB lineage.

Based on this finding, omicron 3 can be considered the progenitor of the omicron lineage 4 and 5 evolution, and their sublineages BA.2.75 (centaurus) and XBB variants.

BQ.1 and BA.5.2.1.7 (BF7) lineages evolved from BA.5. The variant BA.5.2.1.7 spread very fast in China at the end of September 2022 and then in other counties [30, 31]. The wave of infection of BA.5.2.1.7 rapidly led to the accumulation of new mutations (Table 2), diverging rapidly from BQ.1. The latest variants BQ.1.18, also named CH.1.1 or orthrus derived from BQ.1; about its possible origin, the spike deletion Y144 occurred, it is also present in BA.2.12.1 (omicron 3), suggesting that it could originate from patients co-infected with two different variants: BA.2.12.1 and BQ.1.

Regarding the XBB lineage, it has been reported that the XBB variant derives from the recombination of BA.2.10.1 (BJ.1) and BA.2.75 [27], alternatively as a derivative of BA.5 [32]. Analysis of omicron sequence alignment (see supplemental) and the unique mutations presented by the omicron variants (Table 2) revealed that the origin of XBB is from BA.2.10.1, in recombination with BA.2.75.

Again, it has been reported that XBF variants derives from the recombination of between BA.5 and BA.2.75 or BQ variants [29]. Our sequence analysis, by considering the mutation present on the spike protein of XBF, showed a different scenario. Among the mutations, we observed five mutations present in BA.2.75: K147E, W152R, F157L, I210V and G446S; one mutation present in BQ.1: N460K; four mutations present in XBB: G339H, R346T, F486P and F490S, and a unique mutation present only in XBF: G257E (Table 2). This finding supports the origin of XBF as a recombination between BA.2.75 and XBB (Figure 2).

4. Discussion

Several studies have reported that all the new variants originating from SARS-CoV-2 are characterized by the presence of an increased number of mutations that favor a greater viral transmissibility, as well as the virus ability to escape the immune response of the host. In the RBD domain of the omicron's spike protein have been identified nine mutations that affect the binding affinity between viral spike and ACE-2 host receptor, and they are: K417N, G446S, S477N, E484A, Q493R, G496S, Q498R, N501Y, and Y505H [33]. In particular, the mutations S477N, Q498R and N501Y increase binding affinity, representing the molecular basis of the higher virus transmissibility and immune evasion. There are data indicating that BA.2 was 30 % more infectious than BA.1 [34], and a further increase in virus transmission was associated with F486V and L452R mutations in the spike protein of BA.4/5 variants [34].

In the omicron variants, two aspects seem to be related: less severe disease and decreased mortality. Several studies reported that the omicron variants are less dangerous than the previous one, with a reduction in severe disease and hospitalization of patients [35, 36]. Data from England and South Africa, for omicron infected patients, indicated that the probability of requiring hospital treatment was reduced to less than half compared with delta variant infection [34]. Notably, only about 30% of patients developed pneumonia symptoms, and 70 % of them showed mild disease [34].

SARS-CoV-2 use two mechanisms to infect the host cells: by a cell surface fusion process mediated by the TransMembrane Serine PRotease 2 (TMPRSS2), and by the endosomal fusion process mediated by cathepsin [11]. It has been described that wild type and early variants of SARS-CoV-2, like delta variant, were able to infect cells expressing

TMPRSS2, such as lung, alveolar, and gut epithelial cells [11]. On the contrary, the omicron variants, having mutations in the cleavage region of TMPRSS2protease, use the cathepsin-dependent endosomal fusion mechanism, which results in a modified cell tropism towards nasal airway epithelial cells, exhibiting poor TMPRSS2 expression [11]. These are the reasons why delta variant is four-folds more efficient to use the TMPRSS2 mechanism to enter in the cells compared to the omicron variants, but they are ten-fold more efficient to use the endosomal mechanism to enter in the cells, respect the delta variant [11].

Moreover, *in vitro* studies suggested that, in order to infect the nasal epithelium, SARS-CoV-2 used the ACE-2 receptor present on the motile cilia as tracks to penetrate the cell body, by-passing the airway barrier of mucus [37].

Since omicron do not efficiently use the TMPRSS2 system, it does not form syncytia between the host cells, drastically reducing cell-cell direct virus transmission [11]. This different cell tropism of the omicron variants could explain their increased transmissibility over other VOCs, and their reduced danger due to their reduced ability to infect lung epithelial cells, compared to delta and other early variants [11].

The new emerged SARS-CoV-2 omicron sub-lineages, indicated as BQ and XBB, have accumulated additional spike mutations as reported in Table 2, which continue to increase their immune evasion of vaccine. However, recent studies regarding the binding affinity of spike protein in the BQ and XBB sub-lineages, for the ACE-2 receptor was substantially unchanged respect the previously variants whereby their dominance may be mainly due to their advantage in evading antibodies [27].

As suggested by the phylogenetic tree in Figure 2, the last variants, despite the unofficial name, are not phylogenetically unrelated but they are part of BQ and XBB sub-lineages (Figure 2). In some cases, the origin of new variants can take place by a recombination event between two variants, like the variant XBF, originated by the recombination between BA.2.75 and XBB (Table 2, Figure 2).

5. Conclusions

The continue spreading of the last less dangerous omicron variants, showing symptoms and mortality levels comparable to other seasonal flus, suggests a transition in SARS-CoV-2 evolution from pandemic to endemic. Thus, the question is: is it justified to keep the alarm levels against SARS-CoV-2 still so high? The story of SARS-CoV-2 is not over and therefore it is justified to keep alert levels, because there are places in the world where vaccination levels have been very low, thus exposing the population to the risk of new waves of infection. Considering the high mutation rate of SARS-CoV-2, we cannot exclude the onset of new mutations that can completely elude vaccines or generate new and more hazardous variants, for example showing a high mortality.

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References

1. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat. Rev. Microbiol, 2021**;** 19(3): 141-154. https://https://doi.org/10.1038/s41579-020-00459-7.
2. Chan JFW, Kok KH, Zhu Z, Chu H, Wang-TO KK, Yuan S, Yuen K-Y. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg. Microbes Infect., 2020; 9(1): 221–236. https://https://doi.org/10.1080/22221751.2020.1719902. eCollection 2020.
3. Huang Y, Yang C, Xu X-F, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacologica Sinica, 2020; 41: 1141–1149. https://https://doi.org/10.1038/s41401-020-0485-4.
4. Mistry P, Barmania F, Mellet J, Peta K, Strydom A, Viljoen IM, James W, Gordon S, Pepper MS. SARS-CoV-2 Variants, Vaccines, and Host Immunity. Front. Immunol., 2022; 12: 809244. https://https://doi.org/10.3389/fimmu.2021.809244.
5. Rochman ND, Wolf YI, Faure G, Mutz P, Zhang F, Koonin EV. Ongoing global and regional adaptive evolution of SARS-CoV-2. Proc. Natl. Acad. Sci. USA, 2021; 118(29): e2104241118. https://https://doi.org/10.1073/pnas.2104241118.
6. Caputo E, Mandrich L. Structural and Phylogenetic Analysis of SARS-CoV-2 Spike Glycoprotein from the Most Widespread Variants. Life (Bassel), 2022; 12(8): 1245. https://https://doi.org/10.3390/life12081245.
7. Caputo E, Mandrich L. SARS-CoV-2: Searching for the missing variants. Viruses, 2022; 14: 2364. https://https://doi.org/10.3390/v14112364.
8. Tegally H, Moir M, Everatt J, Giovanetti M, Scheepers C, Wilkinson E, Subramoney K, Makatini Z, Moyo S, Amoako DG, Baxter C, Christian L. Emergence of SARS-CoV-2 Omicron lineages BA.4 and BA.5 in South Africa. Nat. Med**,** 2022**;** 28: 1785-1790. https://doi.org/10.1038/s41591-022-01911-2.
9. Ferretti L, Wymant C, Kendall M, Zhao L, Nurtay A, Abeler-Dörner L, Parker M, Bonsall D, Fraser C. Quantifying SARS-CoV-2 transmission suggests epidemic control with digital contact tracing. Science, 2020; 368(6491): eabb6936. https://doi.org/10.1126/science.abb693.
10. Caputo E, Mandrich L. SARS-CoV-2 infection: a case family report. Global Journal of Advanced Research, 2021; 8(3): 83-88.
11. Strohl WR, Ku Z, A Z, Carroll SF, Keit BA, Strohl LM. Passive immunotherapy against SARS-CoV-2: from plasma-based therapy to single potent antibodies in the race to stay ahead of the variants. BioDrugs, 2022; 36: 231-323. https://doi.org/10.1007/s40259-022-00529-7
12. Zhang H, Chen W, Ye X, Zhou Y, Zheng Y, Weng Z, Xie J, Zheng K, Su Z, Zhuang X, Yu X. Clinical characteristics of patients infected with novel coronavirus wild strain, Delta variant strain and Omicron variant strain in Quanzhou: A real-world study. Exp. Ther. Med, 2022; 25(1): 62. https://doi.org/10.3892/etm.2022.11761.
13. Xu R, Wang W, Zhang W. As the SARS-CoV-2 virus evolves, should Omicron subvariant BA.2 be subjected to quarantine, or should we learn to live with it? Front. Public Health, 2022; 10: 1039123. https://doi.org/10.3389/fpubh.2022.1039123.

14. Zappa M, Verdecchia P, Angeli F. Knowing the new Omicron BA.2.75 variant ('Centaurus'): A simulation study. Eur. J. Intern. Med, 2022; S0953-6205(22): 00286-2. https://doi.org/10.1016/j.ejim.2022.08.009.
15. Gruell H, Vanshylla K, Tober-Lau P, Hillus D, Sander LE, Kurth F, Klein K. Neutralisation sensitivity of the SARS-CoV-2 omicron BA.2.75 sublineage. The Lancet Infectious Diseases, 2022; 22(10)*:* 1422-1423. https://https://doi.org/10.1016/S1473-3099(22)00580-1.
16. Hirotsu Y, Omata M. Detection of the Omicron BA.2.75 subvariant in Japan. Journal of Infection, 2022; S0163-4453(22): 00523-0. https://doi.org/10.1016/j.jinf.2022.08.038.
17. Shrestha LB, Foster C, Rawlinson W, Tedla N, Bull RA. Evolution of the SARS-CoV-2 omicron variants BA.1 to BA.5: implications for immune escape and transmission. Rev. Med. Virol, 2022; 32: e2381. https://doi.org/10.1002/rmv.2381.
18. Ledford H. How severe are Omicron infections? Nature, 2021; 600(7890): 577-578. https://doi.org/10.1038/d41586-021-03794-8.
19. Lyngse FP, Ortensen MLH, Denwood MJ, Christiansen LE, Moller CH, Skov RL, Spiess K, et al. Household transmission of the SARS-CoV-2 Omicron variant in Denmark. Nat. Commun, 2022; 13: 5573. https://doi.org/10.1038/s41467-022-33328-3.
20. Iketani S, Liu L, Guo Y, Liu L, Chan JF-W, Huang Y, Wang M, Luo, Y, et al. Antibody Evasion Properties of SARS-CoV-2 Omicron Sublineages. Nature, 2022; 604(7906): 553-556. https://doi.org/10.1038/s41586-022-04594-4.
21. Araf Y, Akter F, Tang, YD, Fatemi R, Parvez SA, Zheng C, Hossain G. Omicron variant of SARS-CoV-2: genomics, transmissibility, and responses to current COVID-19 vaccines. J. Med. Virol, 2022; 94(5): 1825-1832. https://doi.org/ 10.1002/jmv.27588.
22. Mistry P, Barmania F, Mellet J, Peta K, Strydom A, Viljoen IM, James W, Gordon S, Pepper MS. SARS-CoV-2 Variants, Vaccines, and Host Immunity. Front. Immunol., 2022; 12: 809244. https://doi.org/10.3389/fimmu.2021.809244.
23. Schmidt, F.; Muecksch, F.; Weisblum, Y.; Da Silva, J.; Bednarski, E.; Cho, A.; Wang, Z.; Gaebler C, Caskey M, Nussenzweig MC, Hatziioannou T, Bieniasz PD. Plasma Neutralization Properties of the SARS-CoV-2 Omicron Variant. N. Engl. J. Med., 2021; NEJMc2119641 2021. https://doi.org/ 10.1056/NEJMc2119641.
24. Nishiura H, Ito K, Anzai A, Kobayashi T, Piantham C, Rodriguez-Morales AJ. Relative reproduction number of SARS-CoV-2 omicron (B.1.1.529) compared with delta variant in South Africa. J. Clin. Med., 2021; 11(1): 30. https://doi.org/ 10.3390/jcm11010030.
25. Desingu PA, Nagarajan K, Dhama K. Emergence of omicron third lineage BA.3 and its importance. J. Med. Virol., 2022; 94(5): 1808-1810. https://doi.org/10.1002/jmv.27601.
26. Tegally H, Moir M, Everatt J, Giovanetti M, Scheepers C, Wilkinson E, Subramoney K, Makatini Z, Moyo S, Amoako DG, Baxter C, Christian L. Emergence of SARS-CoV-2 Omicron lineages BA.4 and BA.5 in South Africa. Nat. Med., 2022; 28*:* 1785-1790. https://doi.org/10.1038/s41591-022-01911-2.
27. Wang Q, Iketani S, Li Z, Liu LM, Guo Y, Huang Y, Bowe, AD, Liu M, Wang M, Yu J, Valdez R, Lauring AS, Sheng Z, Wang HH, Gordon A, Liu L, Ho DD. Alarming antibody evasion properties of rising SARS-CoV-2 BQ and XBB subvariants. Cell, 2022; 186: 1-8. https://https://doi.org/10.1016/j.cell.2022.12.018.
28. Yue C, Song W, Wang L, Jiang F, Chen X, Gao F, She Z, Wang Y, Wang X, Cao Y. ACE-2 binding and antibody evasion I enhanced transmissibility of XBB.1.5. The Lancet, 2023; 23(3): 278-280. https://doi.org/10.1016/S1473-3099(23)00010-5.
29. Akerman A, Milogiannakis V, Jean T, Esneau C, Ruis Silva M, Ison T, et al. Emergence and antibody evasion of BQ, BA.2.75 and SARS-CoV-2 recombinant sub-lineages in the face of maturing antibody breadth at the population level. eBioMedicine, 2023; 90: 104545. https://https://doi.org/10. 1016/j.ebiom.2023. 104545.
30. Mallapaty S. Can China avoid a wave of deaths if it lifts strict zero COVID policy? Nature, 2022; 612: 203. https://doi.org/10.1038/d41586-022-04235-w.
31. Chenchula S, Amarneni KC, Ghanta MK, Padmavathi R, Chandra MB, Adusumilli MB, Chaavan M, Mudda S, Gupta R, Lakhawat B. Clinical Virology and Effect of Vaccination and Monoclonal Antibodies against SARS-CoV-2 Omicron Sub Variant BF.7 (BA.5.2.1.7): A systematic review. MedRxiv, 2022; 12.25.22283940. https://doi.org/10.1101/2022.12.25.22283940.
32. Kurhade, C.; Zou, J.; Xia, H.; Liu, M.; Chang, H.C.; Ren, P.; Xie, X.; Shi, P.-Y. Low neutralization of SARS-CoV-2 omicron BA.2.75.2, BQ.1.1, and XBB.1 by parental mRNA vaccine or a BA.5-bivalent booster. Nature Medicine, 2023; 29(2): 344-347. https://https://https://doi.org/10.1038/s41591-022-02162-x.
33. Dejnirattisai W, Huo J, Zhou D, Zahradnıḱ J, Supasa P, Liu C, et al. SARS- CoV-2 Omicron-B.1.1.529 Leads to Widespread Escape from Neutralizing Antibody Responses. Cell, 2022; 185(3): 467–84.e15. https://doi.org/10.1016/ j. cell.2021.12.046.
34. Xu A, Hong B, Lou F, Wang S, Li W, Shafqat A, An X, Zhao Y, Song L, Tong Y, Fan H. Sub-lineages of the SARS-CoV-2 omicron variants: characteristics and prevention. MedComm, 2022; 3: e172. https://doi.org/1002/mco2.172.
35. Wolter N, Jassat W, Walaza S, Welch R, Moultrie H, Groome M, et al. Early Assessment of the Clinical Severity of the SARS-CoV-2 Omicron Variant in South Africa: A Data Linkage Study. Lancet, 2022; 399(10323): 437–46. https://doi.org/ 10.1016/S0140-6736(22)00017-4.
36. Maslo C, Friedland R, Toubkin M, Laubscher A, Akaloo T, Kama B. Characteristics and Outcomes of Hospitalized Patients in South Africa During the COVID-19 Omicron Wave Compared with Previous Waves. JAMA, 2022; 327(6): 583–584. https://doi.org/10.1001/jama.2021.24868.
37. Wu C-T, Lidsky PT, Xiao Y, Chen R, Lee IT, Nakayama T, Jiang S, He W, Demeter J, Knight MG, Turn RE, Rojas-Hernandez LS, Ye C, Chiem K, Shon J, Martinez-Sobrido L, Bertozzi CR, Nolan GP, Nayak JV, Milla C, Andino R, Jackson PK. SARS-CoV-2 replication in airway epithelia requires motile cilia and microvillar reprogramming. Cell, 2023; 186: 112-130. https://doi.org/10.1016/j.cell.2022.11.030.