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### Virus Research



# Mutational cascade of SARS-CoV-2 leading to evolution and emergence of omicron variant

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### ABSTRACT

*Background:* Emergence of new variant of SARS-CoV-2, namely omicron, has posed a global concern because of its high rate of transmissibility and mutations in its genome. Researchers worldwide are trying to understand the evolution and emergence of such variants to understand the mutational cascade events.

*Methods*: We have considered all omicron genomes (n = 302 genomes) available till 2nd December 2021 in the public repository of GISAID along with representatives of variants of concern (VOC), i.e., alpha, beta, gamma, delta, and omicron; variant of interest (VOI) mu and lambda; and variant under monitoring (VUM). Whole genome-based phylogeny and mutational analysis were performed to understand the evolution of SARS CoV-2 leading to emergence of omicron variant.

*Results*: Whole genome-based phylogeny depicted two phylogroups (PG-I and PG-II) forming variant specific clades except for gamma and VUM GH. Mutational analysis detected 18,261 mutations in the omicron variant, majority of which were non-synonymous mutations in spike (A67, T547K, D614G, H655Y, N679K, P681H, D796Y, N856K, Q954H), followed by RNA dependent RNA polymerase (rdrp) (A1892T, I189V, P314L, K38R, T492I, V57V), ORF6 (M19M) and nucleocapsid protein (RG203KR).

*Conclusion:* Delta and omicron have evolutionary diverged into distinct phylogroups and do not share a common ancestry. While, omicron shares common ancestry with VOI lambda and its evolution is mainly derived by the non-synonymous mutations.

### 1. Introduction

Throughout the globe resurgence of COVID-19 cases has been linked to the emergence of new variants of concern (https://www.hopkinsmedicine.org/health/conditions-and-diseases/coronavirus/first-and-second-waves-of-coronavirus) (Thakur et al., 2021). Currently, the world is witnessing a new variant namely, omicron which was first reported in South Africa on 24th November 2021 from the specimen collected on 9th November 2021(https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states). On 26th November 2021, World Health Organisation (WHO) assigned omicron to the 'variant of concern' (VOC) category due to its ability to poses a higher risk of reinfection as compared to previously reported variants (https://www.who.int/news/item/26–11–2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern; https://www.who.int/news/item/28–11–2021-upd ate-on-omicron). According to the 1st December 2021 update, omicron is reported in at least 23 countries from five out of six WHO regions, with most cases in Africa and Europe (https://www.cnbc. com/2021/12/01/-

who-says-omicron-has-been-found-in-23-countries-across-the-world. html).

There is a lot of uncertainty surrounding the omicron variant. For its risk assessment, scientists and researchers are investigating the intensity of its spread, extent of its infection, effectiveness of detection methods, therapeutics, and vaccine efficacy (Knoll & Wonodi, 2021; Lipsitch & Dean, 2020; Pegu et al., 2021). The onset of omicron is reported with mild diseases suggests its low or mild severity than its previous counterparts like delta (Ewen Callaway, 2021; E. Callaway & Ledford, 2021).

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Abbreviations: VOC, Variant of concern; VOI, Variant of interest; VUM, Variant under monitoring; NSP, Non-structural protein; UTR, Untranslated region; rdrp, RNA dependent RNA polymerase.

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### K. Bansal and S. Kumar

It is known to have a very high mutation rate with more than 30 mutational changes in its spike protein (Ewen Callaway, 2021) (https://www.who.int/publications/m/item/enhancing-- read-

iness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states)

Globally, high risk of reinfection with omicron variant and its ability to evade vaccine-induced immunity resulting in the emergence of new variants of SARS-CoV-2 (Pulliam et al., 2021). Since COVID-19 inception, researchers have been trying to investigate its origin and evolution (Bansal, Kumar, & Patil, 2021; Singh & Soojin, 2021; Tang et al., 2020). We are currently witnessing a global molecular arms race between SARS-CoV-2 and its preventive therapeutics based on diverse regimes such as DNA, RNA, protein or inactivated whole-virion, etc. (Andreadakis et al., 2020; Corey, Mascola, Fauci, & Collins, 2020; Sharma, Sultan, Ding, & Triggle, 2020). This global crisis can be addressed by a very rapid immunization program worldwide. Moreover, the real-time monitoring of evolutionary cascade of SARS-CoV-2 leading to novel variants is utmost. Earlier investigation of several VOC and VOI suggests some of the crucial mutations for viral survival and high infectivity in humans (Boehm et al., 2021; Kumar & Bansal, 2021; Schmidt et al., 2021). However, mutations giving rise to omicron and intra-omicron genomic diversity are not yet analyzed at a population level.

In the present study, we aim to look for the mutational profile of under-monitoring variants reported till now to understand the emergence of a heavily mutated variant named omicron. Interestingly, whole genome-based phylogeny suggests two major phylogroups PG-I and PG-II. Further, mutational analysis depicted the key role of non-synonymous mutations in the evolution of novel variant. Such genome-wide mutational landscape is required for surveillance and vaccine development.

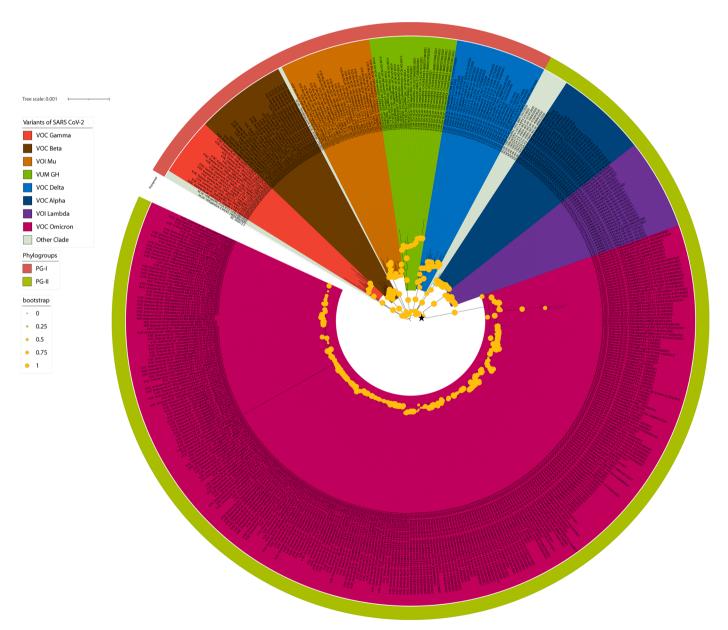


Fig. 1. Maximum likelihood whole genome-based phylogeny of SARS-CoV-2 VOCs, VOIs and VUMs. Here, phylogroups (PG-I and PG-II) and clades (alpha, beta, gamma, delta, omicron, mu etc.) are marked with respective colors as indicated. Bootstrap values are represented by the radius of circle at the nodes. Common ancestry of omicron and lambda is marked by black star.

Table 1

Metadata of the VOCs, VOIs and VUMs strains used in the present study.

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### K. Bansal and S. Kumar

### Table 1 (continued)

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#### Table 1 (continued)

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Delta	bCoV-19/USA/TX-HMH-MCoV-69501/2020	VDC Delta hCoV-19/USA/TX-HMH-MCoV-49501/2020	betacoron EPI ISL 51	13/7/2020 North Amr USA Tes	seas Houston	North Ame USA	Texas penome	29783 Human	unknown unknown AY.122 GK	Houston & Houston & Randall J. Chttps://ww	20/10/2021
Delta	hCoV-19/USA/TX-HMH-MCoV-48794/2020	VDC_belta_hCoV-19/USA/TX-HMH-MCoV-4830()2020	betacoron EPI_ISL_52				Texas penome	29783 Human	unknown unknown AY.100 GK	Houston & Houston & Randall J. ( https://ww	20/10/2021
Delta	hCoV-19/USA/WV-WVU-WV121217/2020	VOC_Delta_hC6V-19/USA/WV-WVU-WV121217/2020	betacoron EPI ISL 46				West Virgi genome	29763 Human 29818 Human	67 Male AY 25 GK	United Ho WVU and I James Den https://wv	1/10/2021
Delta	hCoV-19/050/WV-WV0-WV12121/)2020 hCoV-19/indonesia/IA-GS-EUK-RSRM-0168/2020	VDC_Delta_hCoV-19/05/04/44/06/04/12121/2020 VDC_Delta_hCoV-19/indonesia/M-GS-EUK-RSRM-0168/2020	betacoron EPI ISL 52	29/7/2020 Asia Indonesia Jar		Asia Indonesia		29775 Human	50 Male AY.23 GK	Raden Ma: Eikman Rr Sukma Okt https://ww	21/10/2021
Delta	hCoV-19/indonesia/IA-GS-EUK-RSRM-0168/2020 hCoV-19/indonesia/IA-GS-EUK-RSRM-0169/2020	VOC_Delta_hCoV-19/indonesia/IA-GS-EUK-KSRM-0168/2020 VOC_Delta_hCoV-19/indonesia/IA-GS-EUK-RSRM-0169/2020	betacoron EPI_ISL_52 betacoron EPI_ISL_52	29/7/2020 Asia Indonesia Jar 29/7/2020 Asia Indonesia Jar		Asia Indonesia Asia Indonesia		29775 Human 29769 Human	7 Male AY.23 GK	Raden Ma: Eijkman Re Lydia V Pai https://wv Raden Ma: Eijkman Re Lydia V Pai https://wv	21/10/2021
Delta	hCoV-19/indonesia/IA-GS-EUK-RSRM-0109/2020 hCoV-19/indonesia/IA-GS-EUK-RSRM-0170/2020	VOC_Delta_hC6V-19/indonesia/IA-GS-EUK-KSRM-0169/2020 VOC_Delta_hC6V-19/indonesia/IA-GS-EUK-RSRM-0170/2020	betacoron EPI_ISL_52 betacoron EPI_ISL_52	29/7/2020 Asia Indonesia Jan 29/7/2020 Asia Indonesia Jan	mbi Jambi	Asia Indonesia		29769 Human 29769 Human	39 Male AY 24 GK	Raden Ma: Eikman Rr Willy Agus https://ww	21/10/2021
Delta	hCoV-19/indonesia/IA-GS-EUK-RSRM-0170/2020 hCoV-19/indonesia/IA-GS-EUK-RSRM-0172/2020	VOC_belta_hCoV-19/indonesia/JA-GS-EUK-RSRM-0170/2020 VOC_belta_hCoV-19/indonesia/JA-GS-EUK-RSRM-0172/2020	betacoron EPI_ISL_52	29/7/2020 Asia Indonesia Jar 29/7/2020 Asia Indonesia Jar		Asia Indonesia		29769 Human 29769 Human	46 Female AY.23 GK	Raden Ma: Eikman Rr Jessica R S https://ww	21/10/2021
Delta	hCoV-19/indonesia/IA-GS-EUK-RSRM-0172/2020 hCoV-19/indonesia/IA-GS-EUK-RSRM-0200/2020	VDL_Delta_hCoV-19/indonesia/IA-GS-EUK-KSRM-01/2/2020 VDC Delta hCoV-19/indonesia/IA-GS-EUK-RSRM-0200/2020	betacoron EPI_ISL_5: betacoron EPI_ISL_52	29/7/2020 Asia Indonesia Jar 29/7/2020 Asia Indonesia Jar		Asia Indonesia Asia Indonesia		29769 Human 29769 Human	46 Female AY.23 GK 32 Female AY.23 GK	Raden Ma: Eijkman Re Jessica R S https://ww Raden Ma: Eijkman Re Sukma Okt https://ww	21/10/2021
Delta	hCoV-19/indonesia/JA-GS-EUK-RSKM-0200/2020 hCoV-19/England/PHEP-YYBYUTJ/2020	VOC_Delta_hCoV-19/indonesia/W-GS-EUK-KSKM-0200/2020 VDC_Delta_hCoV-19/England/PHEP-YYBYUTI/2020	betacoron EPI ISL 52	3/8/2020 Europe United Kin En		Asia Indonesia Europe United Kin		29769 Human 29769 Human	unknown unknown AY.4 GK	Respirator COVID-19 PHE Covid https://ww	18/10/2021
Delta	hCoV-19/USA/CA-LACPHL-AF03229/2020	VDC_Delta_hCoV-19/England/PhEP-THT011/2020 VDC_Delta_hCoV-19/USA/CA-LACPHL-AF03229/2020	betacoron EPI_ISL_SC		ilifornia Los Angele		England genome California genome	29769 Human 29598 Human	unknown unknown AY.3 GK	Torrance F Los Angele P. Hemara https://wv	12/10/2021
						North Ame USA					
Delta	hCoV-19/USA/VI-Yale-10210/2020	VOC_Delta_hCoV-19/USA/VI-Yale=10210/2020 VOC Delta hCoV-19/USA/VI-Yale=10211/2020	betacoron EPI_ISL_45				US Virgin I genome	29775 Human 29775 Human	unknown unknown B.1.617.2 GK	VIDOH (V) Grubaugh Joseph Fai, https://wv	29/9/2021 29/9/2021
Delta	hCoV-19/USA/VI-Yale-10211/2020		betacoron EPI_ISL_45	21/8/2020 North Ame USA US		North Ame USA	US Virgin I genome	29775 Human 29835 Human	unknown unknown AY.35 GK unknown unknown AY.82 G	VIDOH (V) Grubaugh Joseph Fau https://ww	
	hCoV-19/Fiji/Fi493/2020	VDC_Delta_hCoV-19/Fij/FJ493/2020	betacoron EPI_ISL_48	25/8/2020 Oceania Fiji		Oceania Fiji	genome			Fiji Centre Microbiolc Sahukhan, https://wv	4/10/2021
Delta	hCoV-19/USA/CA-SEARCH-109796/2020	VDC_Delta_hCoV-19/USA/CA-SEARCH-109795/2020	betacoron EPI_ISL_S2		ilifornia San Diego		California genome	29804 Human	unknown unknown B.1.617.2 GK	San Diego Andersen I SEARCH AI https://ww	15/10/2021
Delta	hCoV-19/USA/WY-WYPHL-21070660/2020	VDC_Delta_hCoV-19/USA/WY-WYPHL-21070660/2020	betacoron EPI_ISL_45			North Ame USA	Wyoming genome	29818 Human	10 Male AY.3 GK	Wyoming Wyoming I Jim Milder https://wv	28/9/2021
Delta	hCoV-19/india/MP-NCDC-2509230/2020	VOC_Delta_hCoV-19/india/MP-NCDC-2509230/2020	betacoron EPI_ISL_24				Madhya Pi genome	29778 Human	63 Male AY.122 GK	National C NCDC Delh Radhakrish https://ww	9/6/2021
Delta	hCoV-19/USA/ND-NDDH-4397/2020	VDC_Delta_hCoV-19/USA/ND-NDDH-4397/2020	betacoron EPI_ISL_48	9/9/2020 North Ame USA No	orth Dakota	North Amc USA	North Dak genome	29775 Human	unknown unknown AY.44 GK	North Dak: North Dak: Lisa Winge https://wv	4/10/2021
Delta	hCoV-19/Rwanda/CV2197/2020	VOC_Delta_hCoV-19/Rwanda/CV2197/2020	betacoron EPI_ISL_43	10/9/2020 Africa Rwanda Ny	arugenge	Africa Rwanda	Nyarugen; genome	29804 Human	34 Male B.1.617.2 GK	Rwanda N. Africa Cen/ Enatha Mu https://wv	4/10/2021
Delta	hCoV-19/BurkinaFaso/CV1920/2020	VOC Delta hCoV-19/BurkinaFaso/CV1920/2020	betacoron EPI ISL 42	16/9/2020 Africa Burkina Fa Ou	uoguobagau	Africa Burkina Fa	Ousgadou genome	29842 Human	unknown unknown AY.36 GK	Centre Ho. Africa Cerr Olawoye, I https://wv	17/9/2021
Delta	hCoV-19/BurkinaFaso/CV1921/2020	VOC_Delta_hCoV-19/BurkinaFaso/CV1921/2020	betacoron EPI_ISL_42	18/9/2020 Africa Burkina Fa Ou	uagadougou	Africa Burkina Fa	Ouagadou genome	29808 Human	unknown unknown AY.36 GK	Centre Ho. Africa Cerr Olawoye, I https://wv	17/9/2021
Delta	hCoV-19/Mexico/AGU-InDRE_FB18599_54467/2020	VDC_Delta_hCoV-19/Mexico/AGU-inDRE_FB18599_54467/2020	betacoron EPI ISL 25	22/9/2020 North Ame Mexico Aa	ruascalientes	North Ame Mexico	Aguascalic genome	29814 Human	22 Female AY.100 GK	LESP Agua Instituto di Claudia Wihttps://wv	14/7/2021
Delta	hCoV-19/india/MH-ICMR-MCL 5716 5772/2020	VOC Delta hCoV-19/india/MH-ICMR-MCL 5716 5772/2020	betacoron EPI ISL 34 2	020-10 Asia India Ma	aharashtra	Asia India	Maharash genome	29873 Human	12 Male AY.50 GK	Indian Co., Indian Cou Pragva Yac https://wv	18/8/2021
Delta	bCoV-19/india/MH-KMR-MCL 5715 5771/2020	VOC Delta hCoV-19/india/MH-ICMR-MCL 5715 5771/2020	betacoron EPI ISL 34 2	020-10 Asia India Ma	abarashtra	Asia India	Maharash eenome	29886 Human	17 Female B.1.617.2 GK	Indian Cos Indian Cou Pragya Yac https://ww	18/8/2021
Delta	hCoV-19/France/PAC-IHU-38688-NovaE/2020	VOC Delta hCoV-19/France/PAC-IHU-38688-NovaE/2020	betacoron EPI ISL 63 2		ovence-i Marseille	Europe France	Provence- senome	29689 Human	unknown unknown AY.43 GK	MEPHI, Ai: MEPHI, Aix Anthony LI https://wv	16/11/2021
Mu	hCoV-19/Colombia/MAG-INS-VG-482/2021	VDI Mu hCoV-19/Colombia/MAG-INS-VG-482/2021	betacoron EPI_ISL_12	11/1/2021 South Ame Colombia Ma	aedalena	South Ame Colombia		29494 Human	unknown Female B.1.621 GH	Universida Instituto N Katherine   https://ww	11/3/2021
Mu	hCoV-19/USA/CA-HLX-STM-000080848/2021	VOI Mu hCoV-19/USA/CA-HLX-STM-000080848/2021	betacoron EPI ISL 24	2021 North Ame USA Ca	lifornia		California genome	29902 Human	unknown unknown 8.1.621 GH	Helix Helix Celina And https://ww	21/6/2021
Mu	hCoV-19/England/PHEC-31028C/2021	VOI Mu hCoV-19/England/PHEC-31028C/2021	betacoron EPI ISL 25	2021 Europe United Kin En		Europe United Kin		29819 Human	unknown unknown B.1.621 GH	Respirator COVID-19 PHE Covid https://ww	30/6/2021
Mu	hCoV-19/England/PHEC-313887/2021	VDL_Mu_hCoV-19/England/PHEC-313887/2021	betacoron EPI_ISL_23	2021 Europe United Kin En		Europe United Kin		29818 Human	unknown unknown B.1.621.1 GH	Respirator COVID-19 PHE Covid https://ww	30/6/2021
Mu	hCoV-19/England/PHEC-L306LE1C/2021	VOLMu_hCoV-19/England/PHEC-L306LE1C/2021	betacoron EPI_ISL_23	2021 Europe United Kin En		Europe United Kin		29835 Human	unknown unknown B.1.621.1 GH	Respirator COVID-19 PHE Covid https://ww	30/6/2021
Mu	hCoV-19/England/PHEC-L306LE3A/2021	VOLMU_ICOV-19/England/PHEC-L306LE3A/2021 VOLMU_hCoV-19/England/PHEC-L306LE3A/2021	betacoron EPI ISL 23	2021 Europe United Kin En		Europe United Kin		29834 Human	unknown unknown 8.1.621.1 GH	Respirator COVID-19 PHE Covid https://wv Respirator COVID-19 PHE Covid https://wv	30/6/2021
Mu	hCoV-19/England/PHEC-L306LE39/2021 hCoV-19/Northernireland/PHEC-L307L689/2021	VOL_MU_RC8V-19/England/PHEC-L306LE3/V2021 VOL Mu_RC8V-19/Northernireland/PHEC-L307L689/2021	betacoron EPI_ISL_23	2021 Europe United Kin No 2021 Europe United Kin No			Northern Leenome	29839 Human 29830 Human	unknown unknown 8.1.621.1 GH	Respirator COVID-19 PHE Covid https://ww Respirator COVID-19 PHE Covid https://ww	30/6/2021
								29747 Human			
Mu	hCoV-19/USA/FL-Shands-VTM-1545/2020	VOL_Mu_hCoV-19/USA/FL-Shands-VTM-1545/2020 VOL Mu_hCoV-19/USA/GA-GPHL-0609/2021	betacoron EPI_ISL_25 2 betacoron EPI_ISL_31				Florida genome	29747 Human 29835 Human	unknown unknown B.1.621.1 GH	UF Health Salemi Lab Magalis BF https://ww	19/7/2021 27/7/2021
	hCoV-19/USA/GA-GPHL-0609/2021						Georgia genome		unknown unknown B.1.621 GH	GA Depart GA Depart Stacy Reev https://wv	
Mu	hCoV-19/USA/GA-GPHL-0611/2021	VDI_Mu_hCoV-19/USA/GA-GPHL-0611/2021	betacoron EPI_ISL_31				Georgia genome	29835 Human	unknown unknown B.1.621 GH	GA Depart GA Depart Stacy Reev https://wv	27/7/2021
Mu	hCoV-19/Venezuela/Fal1944/2021	VDI_Mu_hCoV-19/Venezuela/Fal1944/2021	betacoron EPI_ISL_35	21/1/2021 South Ame Venezuela Fal		South Ame Venezuela		29831 Human	unknown unknown B.1.621 GH	Instituto V Laboratori Rossana C https://ww	19/8/2021
Mu	hCoV-19/France/IDF-CER8AHC-0767669/2021	VDI_Mu_hCoV-19/France/IDF-CERBAHC-0767669/2021	betacoron EPI_ISL_31				Ile-de-Frar genome	29822 Human	28 Female B.1.621 GH	LBM Porte CERBA Hei Bénédicte https://wv	20/8/2021
Mu	hCoV-19/France/OCC-CERBAHC-0737599/2021	VOI_Mu_hCoV-19/France/OCC-CERBAHC-0737599/2021	betacoron EPI_ISI_35				Occitanie genome	29817 Human	15 Female B.1.621 GH	LBM Cerbi CERBA Hei Bénédicte https://wv	20/8/2021
Mu	hCoV-19/England/PHEC-345E6C/2021	VOI_Mu_hCoV-19/England/PHEC-345E6C/2021	betacoron EPI_ISL_35	2021 Europe United Kin, En			England genome	29525 Human	unknown unknown B.1.621 GH	Respirator COVID-19 PHE Covid https://wv	23/8/2021
Mu	hCoV-19/France/IDF-CER8AHC-0786809/2021	VDI_Mu_hCoV-19/France/IDF-CERBAHC-0786809/2021	betacoron EPI_ISL_33	2021 Europe France Ile	-de-France	Europe France	lle-de-Frar genome	29843 Human	23 Female B.1.621 GH	EYLAU - UI CERBA Hei Bénédicte https://wv	26/8/2021
Mu	hCoV-19/France/CVL-CERBAHC-08428324/2021	VOI_Mu_hCoV-19/France/CVL-CER8AHC-08428324/2021	betacoron EPI_ISL_38	2021 Europe France Ce	entre-Val de Loire	Europe France	Centre-Val genome	29817 Human	6 Female B.1.621 GH	Laboratoir CERBA He: Bénédicte https://wv	2/9/2021
Mu	hCoV-19/France/NAQ-CERBAHC-09538751/2021	VOI_Mu_hCoV-19/France/NAQ-CERBAHC-09538751/2021	betacoron EPI ISL 41	2021 Europe France No	ouvelle-Aquitaine	Europe France	Nouvelle-7 genome	29827 Human	28 Female B.1.621.1 GH	Cerballian CERBA Hei Bénédicte https://wv	15/9/2021
Mu	hCoV-19/Colombia/VAC-UTP-VG-024/2021	VDI_Mu_hCoV-19/Colombia/VAC-UTP-VG-024/2021	betacoron EPI_ISL_SE	2021 South Ame Colombia Va	ille del Cauca	South Ame Colombia	Valle del C genome	29781 Human	unknown Male B.1.621 GH	SYNLAB At Laboratori Fredy A. Tahttps://ww	27/10/2021
Mu	hCoV-19/Colombia/VAC-UTP-VG-026/2021	VOI Mu hCoV-19/Colombia/VAC-UTP-VG-026/2021	betacoron EPI ISL 56	2021 South Ame Colombia Va	ille del Cauca	South Ame Colombia	Valle del C genome	29781 Human	unknown Female B.1.621 GH	SYNLAB ÁI Laboratori Fredy A. Tc https://wv	27/10/2021
Mu	hCoV-19/Colombia/VAC-UTP-VG-027/2021	VOI Mu hCoV-19/Colombia/VAC-UTP-VG-027/2021	betacoron EPI ISL SE	2021 South Ame Colombia Va	ille del Cauca	South Ame Colombia	Valle del C senome	29781 Human	unknown Female B.1.621 GH	SYNLAB At Laboratori Fredy A. Tahttps://ww	27/10/2021
Mu	hCoV-19/Colombia/VAC-UTP-VG-029/2021	VDI_Mu_hCoV-19/Colombia/VAC-UTP-VG-029/2021	betacoron EPI_ISL_SE	2021 South Ame Colombia Va		South Ame Colombia		29775 Human	unknown Female B.1.621 GH	SYNLAB Át Laboratori Fredy A. Ti https://ww	27/10/2021
Mu	hCoV-19/Colombia/VAC-UTP-VG-025/2021	VDI_Mu_hCoV-19/Colombia/VAC-UTP-VG-025/2021	betacoron EPI_ISL_SE	2021 South Ame Colombia Va		South Ame Colombia		29778 Human	unknown Male B.1.621 GH	SYNLAB AT Laboratori Fredy A. Ta https://ww	27/10/2021
Mu	hCoV-19/Colombia/VAC-UTP-VG-228/2021	VOI Mu hCoV-19/Colombia/VAC-UTP-VG-228/2021	betacoron EPI ISL 56	2021 South Ame Colombia Va		South Ame Colombia		29781 Human	unknown Female 8.1.621 GH	SYNLAB AT Laboratori Fredy A. T: https://ww	27/10/2021
Mu	hCov-19/USA/IN-LH000111869/2021	VOI Mu hCov-19/USA/IN-LH000111869/2021	betacoron EPI ISL 55				Indiana eenome	29799 Human	38 Female B.1.621 GH	PPHC-Pure Animal Dis Rebeccoa i https://ww	29/10/2021
Mu	hCoV-19/Colombia/ANT-LDSP461/2020	VOI Mu hCoV-19/Colombia/ANT-LOSP461/2020	betacoron EPI ISL 65	14/10/2020 South Ame Colombia An		South Ame Colombia		29781 Human	89 Male B.1.621 GH	SOMER Laboratori Idabely Be https://ww	19/11/2021
Mu	hCoV-19/colombia/ART-CLSP461/2020 hCoV-19/indonesia/IA-FUK-RSRM-175/2021	bCoV-19/Indopesia/IA-FIIK-RSRM-125/2021	betacoron EPI ISL 31	18/5/2021 Asia Indonesia Iar		Asia Indonesia		29781 Human 29782 Human	18 Female B 1640 GH	RSUD Rady Elikman In: Sukma Okt https://ww	1/8/2021
GH	hCoV-19/indonesia/JA-EUK-HSRM-175/2021 hCoV-19/Contro/FCRM-100-A32/28/09/21/2021		betacoron EPI_ISL_33 betacoron EPI_ISL_55	28/9/2021 Asia Indonesia Jar 28/9/2021 Africa Republic o Bri				29782 Human 29683 Human	18 Female 8.1.640 GH 39 Female 8.1.640 GH	Fondation Fondation Misutou h https://ww	27/10/2021
GH	hCoV-19/Congo/FCRM-100-A32.28.09.21/2021 hCoV-19/Canada/0C-100397423001/2021	VUM_GH_hCoV-19/Congo/FCRM-100-A32-28/09.21/2021 VUM_GH_hCoV-19/Congo/FCRM-100-A32-28/09.21/2021	betacoron EPI_ISL_51 betacoron EPI_ISL_64	28/9/2021 Africa Republic o Bri 2/10/2021 North Ame Canada On		Africa Republic o North Arris Canada	Brazzaville genome	29683 Human 29734 Human	39 Female 8.1.640 GH 34 unknown 8.1.640 GH	Fondation Fondation Miloutou N https://ww Laboratoir Laboratoir Sandrine N https://ww	27/10/2021 18/11/2021
GH	hCoV-19/Switzerland/VD-ETHZ-34362364/2021	VUM_GH_hCoV-19/Switzerland/VD-ETHZ-34362364/2021	betacoron EPI_ISL_55	6/10/2021 Europe Switzerlan Va		Europe Switzerlan		29788 Human	unknown unknown B.1.640 GH	Viollier AG Departmer Christian B https://ww	26/10/2021
GH	hCoV-19/England/PHEC-3M04EMF0/2021	VUM_GH_hCoV-19/England/PHEC-3M04EMF0/2021	betacoron EPI_ISL_54	11/10/2021 Europe United Kin En		Europe United Kin		29779 Human	unknown unknown B.1.640 GH	Respirator COVID-19 PHE Covid https://ww	25/10/2021
GH	hCoV-19/France/BRE-CHURENNES-8600727075/2021	VUM_GH_hCoV-19/France/BRE-CHURENNES-8600727075/2021	betacoron EPI_ISL_SE		etagne Bannalec		Bretagne genome	29799 Human	9 Female B.1.640 GH	Laboratoir CHU Ponte GROLHIER https://ww	28/10/2021
GH	hCoV-19/France/BRE-CHURENNES-8600727092/2021	VUM_GH_hCoV-19/France/BRE-CHURENNES-8600727092/2021	betacoron EPI_ISL_SE		etagne Bannalec		Bretagne genome	29795 Human	9 Male B.1.640 GH	Laboratoir CHU Pontc GROLHIER https://wv	28/10/2021
GH	hCoV-19/France/BRE-CHURENNES-8600727095/2021	VUM_GH_hCoV-19/France/BRE-CHURENNES-8600727095/2021	betacoron EPI_ISL_56		etagne Bannalec	Europe France	Bretagne genome	29821 Human	10 Male B.1.640 GH	Laboratoir CHU Pontc GROLHIER https://wv	28/10/2021
GH	hCoV-19/France/PAC-HCL021184595901/2021	VUM_GH_hCoV-19/France/PAC-HCL021184595901/2021	betacoron EPI_ISL_55		ovence-i Alpes-Mar	Europe France	Provences genome	29728 Human	36 Male B.1.640 GH	LABORATE CNR Virus Antonin Be https://ww	4/11/2021
GH	hCoV-19/France/BRE-CHURENNES-8600727084/2021	VUM_GH_hCoV-19/France/BRE-CHURENNES-8600727084/2021	betacoron EPI_ISL_SE		etagne Bannalec	Europe France	Bretagne genome	29770 Human	10 Male B.1.640 GH	Laboratoir CHU Ponte GROLHIER https://wv	28/10/2021
GH	hCoV-19/France/IDF-CER8AHC-10167435/2021	VUM_GH_hCoV-19/France/IDF-CERBAHC-10167435/2021	betacoron EPI_ISL_55		-de-France	Europe France	lie-de-Frar genome	29822 Human	36 Female B.1.640 G	LBM Porte CERBA He: Bénédicte https://wv	27/10/2021
GH	hCoV-19/Scotland/QEUH-27D6EB1/2021	VUM_GH_hCoV-19/Scotland/QEUH-27D6EB1/2021	betacoron EPI_ISL_56	18/10/2021 Europe United Kin Sci	otland	Europe United Kin	Scotland genome	29434 Human	unknown unknown B.1.640 GH	Lighthouse Wellcome Harper Vachttps://ww	27/10/2021
GH	hCoV-19/France/BRE-CHURENNES-8600727081/2021	VUM GH hCoV-19/France/BRE-CHURENNES-8600727081/2021	betacoron EPI ISL SE			Europe France	Bretagne genome	29770 Human	10 Male B.1.640 GH	Laboratoir CHU Ponte GROLHIER https://ww	28/10/2021
GH	hCoV-19/France/BRE-CHURENNES-8600727096/2021	VUM GH hCoV-19/France/BRE-CHURENNES-8600727096/2021	betacoron EPI ISL 56		etagne Bannalec		Bretagne genome	29829 Human	10 Male B.1.640 GH	Laboratoir CHU Pontc GROLHIER https://wv	28/10/2021
GH	hCoV-19/France/BRE-CHURENNES-8600727078/2021	VUM GH hCoV-19/France/BRE-CHURENNES-8600727078/2021	betacoron EPI ISL 56		etazne Bannalec		Bretagne genome	29733 Human	5 Female 8.1.640 GH	Laboratoir CHU Pontc GROLHIER https://wv	28/10/2021
GH	hCoV-19/Congo/FCRM-115-12574/2021	VUM GH hCoV-19/Conzo/FCRM-115-12574/2021	betacoron EPI ISL 60	19/10/2021 Africa Republic o Bri			Brazzaville genome	29714 Human	32 Female 8.1.640 GH	Fondation Fondation Mfoutou N https://ww	9/11/2021
GH	hCoV-19/France/BRE-CHURENNES-8600727100/2021	VUM GH hCoV-19/France/BRE-CHURENNES-8600727100/2021	betacoron EPI ISL 56				Bretagne genome	29770 Human	12 Female B.1.640 GH	Laboratoir CHU Ponte GROLHIER https://ww	28/10/2021
GH	hCoV-19/Conzo/FCRM-127-A23.20.10.21/2021	VUM_GH_hCoV-19/Conzo/FCRM-127-A23.20.10.21/2021	betacoron EPI ISL 60	20/10/2021 Africa Republic o Bra			Brazzaville genome	29649 Human	4 Female B.1.640 GH	Fondation Fondation Mfoutou h https://ww	9/11/2021
GH	hEoV-19/Erapor/BRF-IP932353/2021	VUM GH hCoV-19/France/BRE-IPP32353/2021	betacoron EPI ISL 64				Bretagne genome	29733 Human	16 Female 8.1.640 GH	Labo Anali National R Marion Ba https://ww	18/11/2021
GH	hCol/19/France/DE_HMN.21112080204/2021	VIIM_GH_hCoV-15/Prance/DE-HMN-21112080204/2021	betacoron EPI_ISL_62				lle-de-Frar genome	29733 Human	26 Male 81.640 GH	Hopital Lo Department Christophe https://ww	12/11/2021
GH	hCoV-19/trance/UP-mini-21112060204/2021 hCoV-19/trane/ 02-IEO-10233054/2021	VIM_GH_hCdV-19/(rande)/07-minik-21112080204/2021 VIIM_GH_hCdV-19/(rande)/02-FO.10233054/2021	betacoron EPI_ISL_62	23/10/2021 Europe Italy La:			Lazio penome	29741 Human 29774 Human	unknown Male B1640 GH	IRCCS San IRCCS Regi Frauke Go https://ww	31/10/2021
GH	hCoV-19/Conro/FCRM-118-01/2021	VIM_GH_ECK-19/Contro/FCRM-118-01/2021	betacoron EPI_ISL_SI	23/10/2021 Europe Tany Ca 23/10/2021 Africa Republic o Bra			Brazzaville genome	29714 Human	43 Female B1640 GH	Fondation Fondation Minutou & https://ww	9/11/2021
GH	hCoV-19/Congo/rCAW-118-K1/2021 hCoV-19/France/DE-CERBAHC-10287496/2021	VUM_GH_EC0V-19/C01g0/PCHW-118-K1/2021 VUM_GH_EC0V-19/France/IDF-CFRBAHC-10287496/2021	betacoron EPI_ISL_SC				lie-de-Frar genome	29814 Human	61 Male 8.1.640 GH	LBM Bois + CERBA Hey Bénédicte https://ww	4/11/2021
GH	hCoV-19/France/IDF-CERBWHC-10287496y2021 hCoV-19/Contro/FCRM-120-A2/2021	VUM_GH_RC0V-19/France/IDF-CERBARIC-1028/A90/2021 VUM_GH_hCoV-19/Convo/FCRM-120-A2/2021	betacoron EPI_ISL_SS betacoron EPI_ISL_6(	25/10/2021 Europe France lie 25/10/2021 Africa Republic p Bri			Brazzaville genome	29683 Human	35 Male 8.1.640 GH	Fondation Fondation Moutou h https://ww	9/11/2021
GH	hCoV-19/Congo/rCovi-120-A2/2021 hCoV-19/France/IDF-IPP31876/2021	VUM_GH_hCoV-19/Enge/IDF-IPP31876/2021	betacoron EPI ISL 63				lie-de-Frar genome	29745 Human	61 Male B.1.640 GH	Labo Analy National B Marion Ba https://ww	16/11/2021
Lambda	hCoV-19/France/IDF-InF51878/2021 hCoV-19/Ecuador/UEES-12976/2021	VOIA_GR_ICOV-15/FranceyOF-19751876/2021 VOI Lambda hCoV-19/Ecuador/UEES-12976/2021	betacoron EPI ISL 16				Guavas genome	29783 Human	unknown unknown C.37 GR	Omics Scie Omics Scie Derly Andr https://ww	19/4/2021
Lambda	hCoV-19/E018007/0EES-129/6/2021 hCoV-19/Peru/UM-INS-8425/2020	VOL_ambda_hCoV-19/Ectadd0//0EES-12976/2021 VOI Lambda_hCoV-19/Peru/LIM-INS-8425/2020	betacoron EPI_ISL_18 betacoron EPI_ISL_55	2021 South Ame Ecuador Gu 21/7/2020 South Ame Peru Lin			Uma genome	29769 Human 29769 Human	38 Male C.37 GR	Laboratori Laboratori Carlos Pad https://ww	5/11/2021
Lambda	hCoV-19/Peru/LIM-INS-8425/2020 hCoV-19/Argentina/INEI096534/2020	VOI_Lambda_hCoV-19/Peru/LIM-INS-8425/2020 VOI_Lambda_hCoV-19/Argentina/INEI096534/2020	betacoron EPI_ISL_55 betacoron EPI_ISL_21	21/7/2020 South Ame Peru Un 8/11/2020 South Ame Argentina Ciu				29769 Human 29792 Human	38 Male C.37 GR 27 Female C.37 GR	Laboratori Laboratori Carlos Pad https://ww Servicio Vi Instituto N Baumeiste https://ww	5/11/2021 19/5/2021
Lambda	hCoV-19/Argentina/INE096534/2020 hCoV-19/Peru/LIM-INS-895/2020	VDI_Lambda_hCoV-19/Argentina/INE096554/2020 VDI_Lambda_hCoV-19/Peru/LIM-INS-895/2020	betacoron EPI_ISL_21 betacoron EPI_ISL_15					29792 Human 29496 Human	27 Female C.37 GR 34 Female C.37 GR	Laboratori Laboratori Carlos Pad https://ww	19/5/2021
Lambda Lambda	hCoV-19/Peru/LIM-INS-895/2020 hCoV-19/Peru/LIM-UPCH-0372/2021	VOL_Lambda_hCoV-19/Peru/LIM-INS-895/2020 VOI Lambda hCoV-19/Peru/LIM-UPCH-0372/2021	betacoron EPI_ISL_15 betacoron EPI_ISL_16	22/12/2020 South Amt Peru Lin 1/1/2021 South Amt Peru Lin			Lima genome Lima genome	29495 Human 29744 Human	34 Female C.37 GR unknown unknown C.37 GR	Laboratori Laboratori Carlos Pad https://ww Instituto d Laboratori Lenin Mati https://ww	11/4/2021 18/4/2021
Lambda	hCoV-19/Peru/SAM-INS-2426/2021	VOI_Lambda_hCoV-19/Peru/SAM-INS-2426/2021	betacoron EPI_ISL_34			South Ame Peru	San Martir genome	29791 Human	22 Male C.37 GR	Laboratori Laboratori Carlos Pad https://ww	14/8/2021
Lambda	hCoV-19/Peru/SAM-INS-2421/2021	VOI_Lambda_hCoV-19/Peru/SAM-INS-2421/2021	betacoron EPI_ISL_34			South Ame Peru	San Martir genome	29786 Human	25 Male C.37 GR	Laboratori Laboratori Carlos Pad https://wv	14/8/2021
Lambda	hCoV-19/Peru/SAM-INS-2419/2021	VDI_Lambda_hCoV-19/Peru/SAM-INS-2419/2021	betacoron EPI_ISL_34			South Ame Peru	San Martir genome	29783 Human	40 Female C.37 GR	Laboratori Laboratori Carlos Pad https://wv	14/8/2021
Lambda	hCoV-19/Peru/LIM-INS-731/2021	VOI_Lambda_hCoV-19/Peru/LIM-INS-731/2021	betacoron EPI_ISL_11	12/1/2021 South Ame Peru Lin		South Ame Peru	Lima genome	29496 Human	47 Female C.37 GR	Laboratori Laboratori Carlos Pad https://ww	4/3/2021
Lambda	hCoV-19/Peru/LIM-INS-734/2021	VDI_Lambda_hCoV-19/Peru/LIM-INS-734/2021	betacoron EPI_ISL_11	12/1/2021 South Ame Peru Lin		South Ame Peru	Lima genome	29496 Human	38 Female C.37 GR	Laboratori Laboratori Carlos Pad https://wv	4/3/2021
Lambda	hCoV-19/Peru/LIM-INS-737/2021	VDI_Lambda_hCoV-19/Peru/LIM-INS-737/2021	betacoron EPI_ISL_11	12/1/2021 South Ame Peru Lin			Lima genome	29496 Human	74 Female C.37 GR	Laboratori Laboratori Carlos Pad https://ww	4/3/2021
Lambda	hCoV-19/Peru/CAL-INS-670/2021	VOI_Lambda_hCoV-19/Peru/CAL-INS-670/2021	betacoron EPI_ISL_11	17/1/2021 South Ame Peru Ca			Callao genome	29901 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Ga https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-418/2021	VOI_Lambda_hCoV-19/Peru/LIM-INS-418/2021	betacoron EPI_ISL_11	17/1/2021 South Ame Peru Lin			Lima genome	29890 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Gar https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-419/2021	VDI_Lambda_hCoV-19/Peru/LIM-INS-419/2021	betacoron EPI_ISL_11	17/1/2021 South Ame Peru Lin			Lima genome	29901 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Ga https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-IN5-420/2021	VDI_Lambda_hCoV-19/Peru/LIM-IN5-420/2021	betacoron EPI_ISL_11	17/1/2021 South Ame Peru Un			Lima genome	29898 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Gar https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-421/2021	VOI_Lambda_hCoV-19/Peru/LIM-INS-421/2021	betacoron EPI_ISL_11	17/1/2021 South Ame Peru Lin	па	South Ame Peru	Lima genome	29895 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Gar https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-423/2021	VOI Lambda hCoV-19/Peru/LIM-INS-423/2021	betacoron EPI_ISL_11	17/1/2021 South Ame Peru Lin	na	South Ame Peru	Lima genome	29899 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Gar https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-424/2021	VOI Lambda hCoV-19/Peru/LIM-INS-424/2021	betacoron EPI ISL 11	17/1/2021 South Amt Peru Lin			Lima penome	29863 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Ga https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-430/2021	VOI Lambda hCoV-19/Peru/LIM-INS-430/2021	betacoron EPI ISL 11	17/1/2021 South Ame Peru Lin			Lima genome	29895 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Ga https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-433/2021	VOI Lambda hCoV-19/Peru/LIM-INS-433/2021	betacoron EPI ISL 11	17/1/2021 South Ame Peru Lin			Lima genome	29901 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Gar https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-434/2021	VDL Lambda_hCoV-19/Peru/LIM-INS-434/2021	betacoron EPI_ISL_11	17/1/2021 South Ame Peru Lin			Lima genome		unknown unknown C.37 GR	Laboratori Laboratori Ronnie Ga https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-436/2021	VOL_Lambda_hCoV-19/Peru/LIM-INS-436/2021 VOL Lambda_hCoV-19/Peru/LIM-INS-436/2021	betacoron EPI ISL 11	17/1/2021 South Ami Peru Lin 17/1/2021 South Ami Peru Lin			Lima penome	29901 Human	unknown unknown C.37 GR	Laboratori Laboratori Ronnie Gar https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-436/2021 hCoV-19/Peru/LIM-INS-437/2021	VOL_Lambda_PLOV-19/Peru/UM-INS-436/2021 VOI Lambda hCoV-19/Peru/UM-INS-437/2021	betacoron EPI_ISL_11 betacoron EPI_ISL_11						unknown unknown C.37 GR	Laboratori Laboratori Ronnie Gai https://ww	1/3/2021
Lambda	hCoV-19/Peru/LIM-INS-437/2021 hCoV-19/Peru/LIM-INS-643/2021	VOI_Lambda_hCoV-19/Peru/LIM-INS-437/2021 VOI_Lambda_hCoV-19/Peru/LIM-INS-443/2021	betacoron EPI_ISL_11 betacoron EPI ISL 11	17/1/2021 South Ame Peru Un 17/1/2021 South Ame Peru Un			Lima genome Lima penome		unknown unknown C.37 GR unknown unknown C.37 GR	Laboratori Laboratori Ronnie Garhttps://wv Laboratori Laboratori Ronnie Garhttps://wv	1/3/2021
Lambda Lambda	hCoV-19/Peru/LIM-INS-643/2021 hCoV-19/Peru/CAL-INS-668/2021	VOI_Lambda_hCoV-19/Peru/LIM-INS-443/2021 VOI_Lambda_hCoV-19/Peru/CAL-INS-668/2021	betacoron EPI_ISL_11 betacoron EPI_ISL_11	17/1/2021 South Ame Peru Lin 18/1/2021 South Ame Peru Ca		South Ame Peru South Ame Peru		29901 Human 29888 Human	unknown unknown C.37 GR unknown unknown C.37 GR	Laboratori Laboratori Ronnie Garhttps://wv Laboratori Laboratori Ronnie Garhttps://wv	1/3/2021 1/3/2021
Lambda	ncov-zo/Peru/CAL-INS-668/2021	vcs_Lambba_ncoV-19/Peru/CAL-INS-668/2021	Detacoron EP1_ISL_11	16/1/2021 South Ami Peru Ca	nao	Souch Ame Peru	Callao genome	29688 Human	unknown unknown C.37 GR	Laboratori Laboratori Konnie Gar https://wv	1/3/2021

### 2. Results

### 2.1. Phylogenomics suggests common ancestry of omicron and lambda variants

Whole genome-based phylogeny (n = 478 genomes) representing VOC (alpha, beta, gamma, delta, and omicron), VOI (mu and lambda) and VUM depicts two major phylogroups PG-I and PG-II (Fig. 1 and Table 1). Here, the reference strain of SARS-CoV-2 (Wuhan-Hu-1, NC\_045512.2) is taken as an outgroup. PG-I has VOC: gamma, beta, and delta; VOI: mu and VUM: GH. Whereas, PG-II includes VOC: alpha, omicron and VOI: lambda. Interestingly, two VOCs, delta and omicron, belong to different phylogroups. Phylogeny depicted that omicron shares a common ancestry with VOI lambda represented by a black asterisk in Fig. 1. Interestingly, three isolates from Italy (EPI\_ISL\_6854346, EPI\_ISL\_6854347, and EPI\_ISL\_6854348) form a diversified sub-lineage among the omicron population. Additionally, EPI\_ISL\_6886594 from Germany is a diversified omicron strain.

### 2.2. Very high non-synonymous mutations give rise to omicron

Mutation is driving the evolution and emergence of new variants of COVID-19 worldwide (Islam et al., 2021; Kumar & Bansal, 2021; Thakur et al., 2021). Availability of genomic resources have enabled the research community in tracking mutational events and linking them to new variants (Mercatelli & Giorgi, 2020; Rambaut et al., 2020). Analysis and routine surveillance from South Africa suggested omicron ability to evade immunity from prior infection as compared to other VOCs (Pulliam et al., 2021). In the present study, we intend to understand the evolution and emergence of omicron by its mutational landscape at population level.

We have performed a mutational analysis with respect to the reference genome of SARS-CoV-2 (NC\_045512.2) (Fig. 2). Total mutations detected in the dataset were 24,189, and omicron genomes constituted 18,261 mutations (supplementary table 1). For all the strains under study, we have calculated the total number of mutations detected (supplementary table 2). Average mutations per genome for the omicron variant were detected to be 60.5. For the limited genomes of VOCs, VOIs

K. Bansal and S. Kumar

Virus Research 315 (2022) 198765

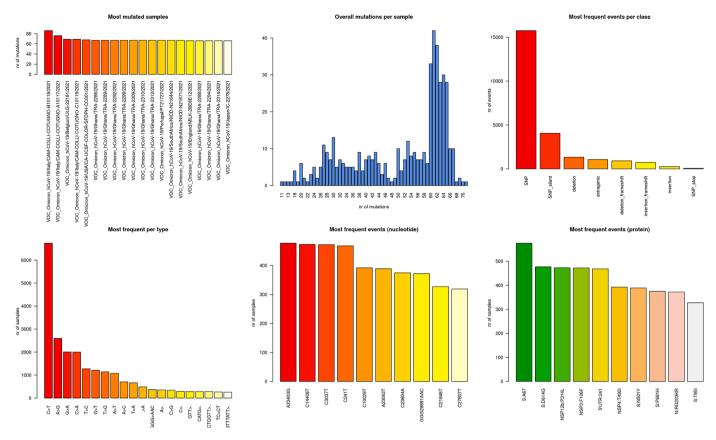


Fig. 2. Mutational analysis of omicron. Six panel image displays the most mutated samples, overall mutations per samples, most frequent events per class of mutation category, changes of nucleotide per type, nucleotide wise most frequent events and protein level most frequent events for the genomes used in the study.

and VUMs, average mutations for GH, delta, mu, gamma, alpha, lambda and beta were 48, 39, 38.5, 37.8, 30.7, 27.4 and 24.2 respectively. This clearly depicts high number of mutations in the omicron variant as compared to other variants of SARS-CoV-2. Except for omicron, average mutations for other variants were calculated on the basis of limited genomes, which might not represent the true mutational events for them. Since, omicron is the recently emerged variant, aim of present study was to understand its mutational landscape at population level.

Interestingly, >97% (n = 17,703 mutations) of the mutations in omicron were in the coding region, and remaining 558 were detected in the extragenic region of the genome. Amongst the coding gene mutations, 2965 were indels while 14,738 were SNPs constituting nonsynonymous (n = 11,995 mutations) and synonymous mutations (n = 2743 mutations). Single nucleotide transitions are shown to be major mutational types amongst the SARS-CoV-2 genomes (Kumar & Bansal, 2021; Mercatelli & Giorgi, 2020).

Interestingly, mutational events are highly skewed towards the spike protein, which constitutes ~60% (n = 10,658) of the total mutations in the coding genomic region (n = 17,703) (Fig. 3). The majority of spike protein mutations encompass A67, T547K, D614G, H655Y, N679K, P681H, D796Y, N856K, Q954H, which are reported in all the omicron genomes analysed (Table 3). Count of mutations in the spike was followed by RNA dependent RNA polymerase (rdrp) (n = 4142) constituting A1892T, I189V, P314L, K38R, T492I, V57V in all omicron genomes analyzed (Fig. 3 and Table 3). Remaining 2903 mutations were detected in rest of the coding genomic region (Table 2, 3, and supplementary table 1), where M19M in ORF6, and RG203KR in nucleocapsid

protein are amongst the most prevalent mutations in omicron (Fig. 3).

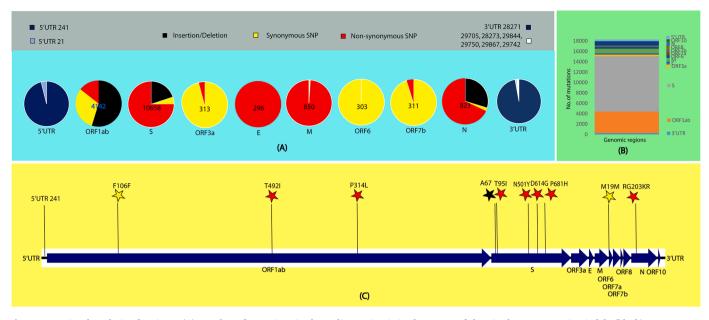
### 2.3. Low intra-sequence diversity amongst omicron variant

Intra-strain diversity among the omicron variant strains reported worldwide will be crucial in understanding the genome dynamics and rapid evolution of SARS-CoV-2. We performed the mutational analysis on the current dataset using omicron (OL677199) isolated from Canada on 23rd November 2021 as the reference genome (supplementary table 3). Most of the strains (n = 298), irrespective of their geographic origin, had less than ten mutations depicting low intra-strain diversity among omicron strains. We found omicron variants had >55 mutations when compared with other VOCs and VOIs. However, four of the isolates two from Europe (Italy) (EPI\_ISL\_6854347 (n = 23 mutations) and EPI\_ISL\_6854346 (n = 14 mutations) and two from South Africa (EPI\_ISL\_6699742 (n = 12 mutations) and EPI\_ISL\_6774091 (n = 11 mutations) were most diversified among the omicron genomes.

### 3. Methods

## 3.1. Identification and procurement of SARS-CoV-2 genome from the public repository

We have considered all the available genomes of omicron variant available in public domain until 6 pm Indian Standard Time (IST) on 2nd December 2021 from GISAID (n = 302 genomes). A total of 25 strains from each variant of concern, namely alpha (B.1.1.7), beta (B.1.351),



**Fig. 3.** Mutational analysis of omicron (A) Number of mutations in the coding region is in the centre of the pie-chart representing indels (black), synonymous (yellow) and non-synonymous (red) SNPs. Type and number of mutations in the extergenic region is represented by pie charts blue, light blue and white as represented in the color legends. (B) Bar graph representing number of mutations in the genomic region of SARS-CoV-2. (C) Some of the top mutations (pl. refer Table 3 for all top mutations in omicron) among the omicron variant are represented by stars of black: indels, yellow: synonymous and red: non-synonymous mutations.

### Table 2

Genomic region wise mutational count of the omicron isolates by taking NC\_045512.2 as a reference.

Genomic region	Mutational count	Annotation
5'UTR	309	5' Untranslated region
NSP1	5	RNA dependent RNA polymerase
NSP2	31	
NSP3	1572	
NSP4	325	
NSP5	317	
NSP6	595	
NSP7	0	
NSP8	2	
NSP9	9	
NSP10	301	
NSP11	0	
NSP12a	0	
NSP12b	632	
NSP13	14	
NSP14	319	
NSP15	6	
NSP16	14	
S	10,658	Spike
ORF3a	313	ORF3a protein
E	296	Envelope
Μ	850	Membrane
ORF6	303	ORF6 protein
ORF7a	2	ORF7a protein
ORF7b	311	ORF7b protein
ORF8	4	ORF8 protein
N	823	Nucleocapsid protein
ORF10	1	ORF10 protein
3'UTR	249	3' Untranslated region

gamma (P.1) and delta (B.1.617.2) and variant of interest, namely lambda (C.37) and mu (B.1.621). We have also considered 25 strains from variant under monitoring, namely GH (B.1.640). These all strains are from their respective earlier reports in the public domain. Pangolin COVID-19 lineage assigner webserver (https://pangolin.cog-uk.io/) was used to truly demarcate the strains of across variants. The investigation suggested that 9 out of 25 strains does not belong to gamma (P.1) and 1 out of 25 strains doesn't belong to VUM GH (B.1.640) and were wrongly classified earlier. A detailed list of all the strains used in the study is provided in Table 1.

### 3.2. Phylogenetic analysis

A total of 477 high-quality genomes, including the major variants spread across the globe were taken into consideration. Multiple sequence alignment was performed for all the genomes using MAFFT v7.467 (Nakamura, Yamada, Tomii, & Katoh, 2018) followed by phylogenetic tree construction using fasttree v2.1.8 with double precision (Price, Dehal, & Arkin, 2010) with gamma time reversal method. Visualization of the obtained phylogenetic tree was performed using iTol v6 (Letunic & Bork, 2019). Different variants were marked in accordance with different colors as mentioned in the legends.

### 3.3. Mutational analysis

Mutational analysis of all the strains (n=477) in the study was performed with two different reference genomes. First with NC\_045512.2 (Wuhan-Hu-1) strain (reference SARS CoV-2 strain) and another with first reported strain of omicron variant (OL677199.1) (https://www. ncbi.nlm.nih.gov/nuccore/OL677199) using nucmer v3.1 (Delcher, Phillippy, Carlton, & Salzberg, 2002). We have used a well-documented R script described earlier (Mercatelli & Giorgi, 2020). Here, we have used gff3 annotation and reference genome file to extract genomic coordinate of SARS-CoV-2 proteins. R library package seqinr (https://cran. r-project.org/web/packages/seqinr/index.html) and biostring package (https://bioconductor.org/packages/release/bioc/html/Biostrings.ht ml) of bioconductor was implemented to obtain the list of all the mutational events. Mutational events were calculated with respect to two different references (Reference SARS CoV-2 strain: NC 045512.2) (https://www.ncbi.nlm.nih.gov/nuccore/NC\_045512.2) and omicron (https://www.ncbi.nlm.nih.gov/nuccore/OL677199) (OL677199.1) separately. Further, the average mutations for a variant were calculated by adding up the mutations in each variant and dividing them by the total number of genomes of the variant used in the present study.

### K. Bansal and S. Kumar

### Table 3

Top mutations (>185 in count) in omicron variant as compared to the reference sequence NC\_045512.2.

annotation	protein	variant	varclass	Count	Refpos	refvar	qvar	qpos	qlengt
Spike	S	A67	deletion_frameshift	575	21,762	С		21,483	29,38
Predicted phosphoesterase, papain-like proteinase	NSP3	A1892T	SNP	302	8393	G	А	8124	29,38
Transmembrane protein	NSP6	I189V	SNP	302	11,537	Α	G	11,259	29,38
RNA-dependent RNA polymerase, post-ribosomal frameshift	NSP12b	P314L	SNP	302	14,408	С	Т	14,130	29,38
pike	S	T547K	SNP	302	23,202	С	Α	22,915	29,38
pike	S	D614G	SNP	302	23,403	Α	G	23,116	29,38
pike	S	H655Y	SNP	302	23,525	С	Т	23,238	29,38
DRF6 protein	ORF6	M19M	SNP_silent	302	27,259	Α	С	26,972	29,38
Predicted phosphoesterase, papain-like proteinase	NSP3	K38R	SNP	301	2832	Α	G	2566	29,38
spike	S	N679K	SNP	301	23,599	Т	G	23,312	29,38
ransmembrane protein	NSP4	T492I	SNP	301	10,029	С	Т	9760	29,37
Nucleocapsid protein	Ν	RG203K*	SNP	301	28,881	GGG	AAT	28,806	29,69
Growth-factor-like protein	NSP10	V57V	SNP silent	300	13,195	Т	С	12,917	29,38
pike	S	P681H	SNP	300	23,604	C	Ā	23,317	29,38
pike	S	D796Y	SNP	300	23,948	G	Т	23,661	29,38
pike	S	N856K	SNP	300	24,130	C	A	23,843	29,38
pike	S	Q954H	SNP	300	24,424	A	Т	24,137	29,38
Jucleocapsid protein	N	RG203KR	SNP	300	28,881	GGG	AAC	28,594	29,38
NA-dependent RNA polymerase, post-ribosomal frameshift	NSP12b	N591N	SNP_silent	298	15,240	C	T	-	29,38
					-			14,962	
pike Iradiatad phasphasetaraga, papain lika proteinasa	S NGD2	T95I	SNP SND silont	298	21,846	C	Т	21,562	29,38
Predicted phosphoesterase, papain-like proteinase	NSP3	F106F	SNP_silent	297	3037	C	T	2771	29,38
pike	S ODE2-	G339D	SNP	297	22,578	G	A	22,291	29,38
DRF3a protein	ORF3a	T64T	SNP_silent	297	25,584	C	Т	25,297	29,38
	5'UTR	241	extragenic	297	241	C	Т	187	29,69
C-like proteinase	NSP5	P132H	SNP	296	10,449	C	A	10,180	29,38
3'-to-5' exonuclease	NSP14	I42V	SNP	296	18,163	А	G	17,885	29,38
Envelope	E	T9I	SNP	296	26,270	С	Т	25,983	29,38
DRF7b protein	ORF7b	L17L	SNP_silent	296	27,807	С	Т	27,520	29,38
pike	S	N969K	SNP	294	24,469	Т	Α	24,182	29,38
redicted phosphoesterase, papain-like proteinase	NSP3	A889A	SNP_silent	293	5386	Т	G	5120	29,38
pike	S	L981F	SNP	292	24,503	С	Т	24,216	29,38
pike	S	D1146D	SNP_silent	292	25,000	С	Т	24,713	29,38
/lembrane	Μ	A63T	SNP	289	26,709	G	Α	26,422	29,38
redicted phosphoesterase, papain-like proteinase	NSP3	S1265	deletion	288	6513	GTT		6246	29,38
ransmembrane protein	NSP6	L105	deletion	287	11,286	TGTCTGGTT		11,016	29,38
Spike	S	168	deletion_frameshift	287	21,767	CATG		21,486	29,38
pike	S	E484A	SNP	284	23,013	Α	С	22,726	29,38
pike	S	S477N	SNP	283	22,992	G	А	22,705	29,38
pike	S	T478K	SNP	283	22,995	С	А	22,708	29,38
pike	S	Q493R	SNP	282	23,040	A	G	22,753	29,38
pike	S	Q498R	SNP	281	23,055	A	G	22,768	29,38
pike	S	N501Y	SNP	281	23,063	A	T	22,776	29,38
pike	S	G496S	SNP	280	23,048	G	A	22,7761	29,38
pike	S	Y505H	SNP	200	23,075	T	C	22,788	29,38
-	M	D3G	SNP	275	26,530	A	G	26,243	29,38
/lembrane					-			-	
Aembrane	M	Q19E	SNP	272	26,577	C	G CT	26,290	29,38
pike Inite	S	S371L	SNP	270	22,673	TC	CT	22,386	29,38
pike	S	S373P	SNP	270	22,679	T	С	22,392	29,38
pike	S	G142	deletion	260	21,987	GTGTTTATT	T	21,702	29,38
pike	S	S375F	SNP	260	22,686	С	Т	22,399	29,38
DRF7b protein	ORF7b	E3*	SNP_stop	253	27,762	G	Т	27,687	29,75
pike	S	I210	insertion_frameshift	243	22,193	•	Т	21,901	29,38
pike	S	R214	insertion_frameshift	243	22,203		Α	21,916	29,38
pike	S	R214R	SNP_silent	243	22,204	Т	А	21,917	29,38
lucleocapsid protein	Ν	E31	deletion	243	28,362	GAGAACGCA		28,074	29,37
pike	S	L212*	SNP_stop	243	22,197	Т	G	22,118	29,74
pike	S	N211K	SNP	242	22,195	Т	G	21,903	29,38
pike	S	L212C	SNP	242	22,197	TA	GC	21,905	29,38
pike	S	S214	insertion	242	22,201		AGC	21,910	29,38
pike	S	V213	insertion frameshift	242	22,202		Α	21,914	29,38
JA	3'UTR	28,271	extragenic	242	28,271	A	Т	27,984	29,37
Nucleocapsid protein	N	P13L	SNP	241	28,311	C	Т	28,024	29,37
Spike	S	N764K	SNP	234	23,854	C	A	23,567	29,38
Spike	S	G446S	SNP	203	23,834 22,898	G	A	23,307 22,611	29,38
-	S			203 199		G T			
Spike Spike		N440K	SNP		22,882		G	22,595	29,38
	S	K417N	SNP	183	22,813	G	Т	22,526	29,38

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### Author contribution statement

Both the authors' KB and SK have contributed equally to the data curation, analysis, and writing of the manuscript.

### CRediT authorship contribution statement

Kanika Bansal: Data curation, Formal analysis, Writing – original draft. Sanjeet Kumar: Data curation, Formal analysis, Writing – original draft.

### **Declaration of Competing Interest**

The author declares no competing interest.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2022.198765.

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