WILEY

Frontiers in Flow Cytometry™ is for researchers across the globe looking for an opportunity to share and learn about current developments in flow cytometry. This 24 hour virtual event will feature keynote presentations by industry colleagues, webinars, demos, live networking opportunities and more.

Key topics include:

- Spectral and conventional flow cytometry
- Immunophenotyping and Standardization
- Panel design and optimization
- Cancer Biology and Auto-immune Diseases
- Infectious diseases
- Advances in flow cytometry technology

Register Now

This event is sponsored by ThermoFisher SCIENTIFIC

DOI: 10.1002/jmv.29075

COMMENTARY

JOURNAL OF MEDICAL VIROLOGY WILEY

Genetic and structural analyses reveal the low potential of the SARS-CoV-2 EG.5 variant

Fabio Scarpa[1] | Stefano Pascarella[2] | Alessandra Ciccozzi[3] | Marta Giovanetti[4,5] | Ilenia Azzena[1,6] | Chiara Locci[1,6] | Marco Casu[6] | Pier Luigi Fiori[1,7] | Miriana Quaranta[2] | Eleonora Cella[8] | Daria Sanna[1] | Massimo Ciccozzi[3]

[1]Department of Biomedical Sciences, University of Sassari, Sassari, Italy

[2]Department of Biochemical Sciences "A. Rossi Fanelli", Sapienza Università di Roma, Rome, Italy

[3]Unit of Medical Statistics and Molecular Epidemiology, Università Campus Bio-Medico di Roma, Rome, Italy

[4]Department of Science and Technology for Humans and the Environment, Università Campus Bio-Medico di Roma, Rome, Italy

[5]Instituto Rene Rachou, Fundação Oswaldo Cruz, Belo, Horizonte, Minas Gerais, Brazil

[6]Department of Veterinary Medicine, University of Sassari, Sassari, Italy

[7]Azienza Ospedaliera Universitaria (AOU) Sassari, Sassari, Italy

[8]Burnett School of Biomedical Sciences, University of Central Florida, Orlando, Florida, USA

Correspondence
Fabio Scarpa, Department of Biomedical Sciences, University of Sassari, Sassari, Italy.
Email: fscarpa@uniss.it

Funding information
FONDAZIONE DI SARDEGNA; PON "Ricerca e Innovazione"; CRP-ICGEB RESEARCH GRANT 2020, Grant/Award Number: CRP/BRA20-03; Sapienza, Grant/Award Number: RP122181671E8B2F

Abstract

The severe acute respiratory syndrome coronavirus 2 EG.5 lineage is the latest variant under monitoring, and it is generating significant concern due to its recent upward trend in prevalence. Our aim was to gain insights into this emerging lineage and offer insights into its actual level of threat. Both genetic and structural data indicate that this novel variant presently lacks substantial evidence of having a high capacity for widespread transmission. Their viral population sizes expanded following a very mild curve and peaked several months after the earliest detected sample. Currently, neither the viral population size of EG.5 nor that of its first descendant is increasing. The genetic variability appear to be flattened, as evidenced by its relatively modest evolutionary rate (9.05×10^{-4} subs/site/year). As has been observed with numerous prior variants, attributes that might theoretically provide advantages seem to stem from genetic drift, enabling the virus to continually adjust to its host, albeit without a clear association with enhanced dangerousness. These findings further underscore the necessity for ongoing genome-based monitoring, ensuring preparedness and a well-documented understanding of the unfolding situation.

Daria Sanna and Massimo Ciccozzi contributed equally to this work.

KEYWORDS
EG.5, genetics, molecular epidemiology, phylodynamics, SARS-CoV-2, virus evolution

During the coronavirus disease 2019 (COVID-19) pandemic, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has experienced a significant amount of genetic mutations, leading to the development of numerous lineages and sub-lineages, each possessing different abilities to spread and expand.[1] As of July 30, 2023, the World Health Organization (WHO) has listed two Variants of Interest (VoIs) (i.e., XBB.1.5 and XBB.1.16) and seven Variants under Monitoring (VuMs) (i.e., BA.2.75, CH.1.1, XBB, XBB.1.9.1, XBB.1.9.2, XBB.2.3, and EG.5) (https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—3-august-2023). Among the VuMs, EG.5 is the most recent and has been detected in 45 countries shown an increasing trend in prevalence from 6.2% in Week 24 to 11.6% by Week 28, while other VuMs decreased (https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—3-august-2023), indicating a higher effective reproduction number compared to neighboring lineages.[2,3] Just as has been carried out previously for other emerging variants that experienced rapid increases in case numbers, it is imperative to conduct a comprehensive in-depth study in this particular case as well. To achieve a comprehensive perspective, we employed an integrative approach that considers both genetic and structural analyses to shed light on this emerging lineage and assess its actual level of dangerousness.

Phylodynamic analyses have been performed following Scarpa et al.[4] by using all available genomes in GISAID portal (https://gisaid.org/) as of August 1, 2023 (EG.5, $n = 713$; EG.5.1, $n = 226$). For details on the analyzed genomes see Supporting Information: File S1. The phylogenomics of SARS-CoV-2 (available at https://gisaid.org/phylodynamics/global/nextstrain/), with a focus on global subsampling of the Omicron GISAID CLADE (Supporting Information: Figure S1), reveals that the genomes of EG.5 (and its descendant EG.5.1) cluster within the GISAID CLADE 23 A (XBB.1.5). Although the sub-lineage EG.5.1 constitute a clade labeled GISAID CLADE 23F, as recently shown in other emerging lineages, such as BF.7 in Asia[5] and XBB on global scale,[4] EG.5 and EG.5.1 are positioned within the main clade as an evolutionary blind background without any direct descendant, with a branch's length denoting the lack of a rapid diversification (see Supporting Information: Figure S1).

Bayesian Skyline Plot (BSP) graph (Figure 1A), reconstructed by following Scarpa et al.,[4] indicated that the genetic variability of the lineages EG.5 and its first descendant EG.5.1 have exhibited very few and mild fluctuations over time, resulting in the expansion and contraction of its viral population size. However, these fluctuations do not coincide with epidemic events and only reflect limited increases in infections. Indeed, the trend of the viral population size is not consistent with the trend shown by the number of cases, which began to increase in the second half of July (see https://cov-spectrum.org/). The common ancestor of all SARS-CoV-2 EG.5 genomes is traced back to February 22, 2023 (interval of confidence: February 8–March 24). This dating is fully consistent with the earliest documented samples of EG.5, which date back to February 17, 2023 (https://www.who.int/activities/tracking-SARS-CoV-2-variants). After its generation, the genetic variability and viral population size of EG.5 experienced a slight increase that lasted for about 2 months, reaching a plateau in the first half of May. The plateau phase persisted until early July, followed by a decrease that led to a subsequent plateau, which has persisted until now. During the mild growth phase of EG.5, its first descendant, EG.5.1, was generated and is dated back to March 13, 2023 (interval of confidence: February 26–April 2). The initial growth of EG.5.1 appeared to be more substantial than that of EG.5, and it peaked after about a month in late April. The plateau phase lasted for less than 2 months, and in late June, both viral population size and genetic variability began to decrease, a reduction that is still persisting. This condition is a typical characteristic of an evolutionary lineage that introduces new features compared to its immediate predecessor, but these new traits, at present, do not represent an additional boost capable of driving an abnormal surge in growth. A similar scenario was observed in mid-2022 with the SARS-CoV-2 variant BA.2.75,[7] which ceased to raise concerns after a few months due to the reduction in its spread. Furthermore, the vicariance (i.e., alternation in terms of prevalence and abundance) observed between EG.5 and EG.5.1 further signifies an ongoing evolution leading to new mutations and subvariants. These developments, from an epidemic perspective, do not raise concerns, as has been observed previously between XBB and XBB.1.[4]

Additionally, the estimated evolutionary rates of EG.5 and EG.5.1 amount to 9.05×10^{-4} (95% HPD: 7.98×10^{-4}–1.02×10^{-3}) and 5.80×10^{-4} (95% HPD: 4.60×10^{-4}–6.88×10^{-4}) substitutions per site per year, respectively. These evolutionary times indicate a limited capacity for significant demographic expansion, and they are consistent with the evolutionary history of the recent sub-variant that initially raised concerns.[8] In addition, it should be noted that the evolutionary rates of EG.5 and EG.5.1 are approximately 10 times lower than that of the original SARS-CoV-2 Wuhan-Hu-1 strain.[9]

All protein structural analyses and modeling have been carried out as described in Pascarella et al.[10] EG.5 and EG.5.1 share the same RBD (Receptor binding domain). The estimated net charge is reported in Table S1. Net charge and distribution of the surface electrostatic potential (Supporting Information: Figure S2) appear similar to that observed in the latest variants.[11] EG.5 and EG.5.1 NTDs (N-terminal domain) differ in the sequence position 52 where Q or H occur, respectively. This difference has a modest effect on the net charge (Supporting Information: Table S1) and surface electrostatic potential

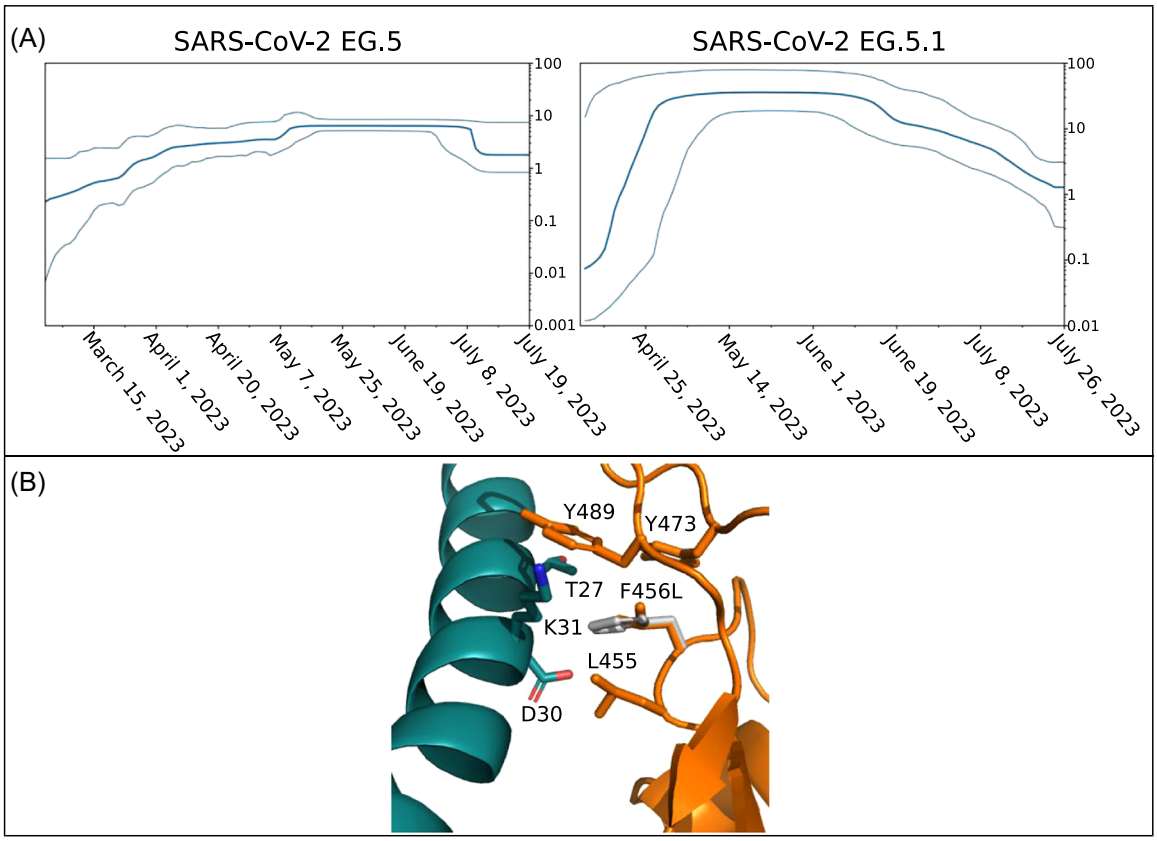

FIGURE 1 (A) Bayesian Skyline Plot of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) EG.5 and EG.5.1 variants. The viral effective population size (y-axis) is shown as a function of days (x-axis). Upper and lower lines represent the 95% high posterior density (HPD) region. These plots have been reconstructed using all of the available genomes as of August 1, 2023 (EG.5, *n* = 713; EG.5.1, *n* = 226). The final run, consisting of 200 million generations, was executed using the Bayesian Skyline Model alongside the uncorrelated log-normal relaxed clock model. The selection of this model was based on a comparison of 2lnBF values derived from marginal likelihoods, as showed in Mugosa et al.[6] See Supporting Information: File S1 for details on the used genomes and Authorship. (B) Ribbon model of SARS-CoV-2 ACE2-RBD interface. ACE2 and RBD are depicted as deep teal and orange models, respectively. The relevant side chains are reported as labeled stick models. The F546L mutant site display the original Phe side chain as a white stick model.

(Supporting Information: Figure S2). The NTD net charge is in the negative region as observed, for example, in XBB.1.5,[11] indicating that the sum total of electric charges within the NTD is negative. A negative NTD electrostatic potential could suggest a reduced capacity to engage with negatively charged cellular elements like sialosides and the AXL receptor.[10] Interestingly, EG.5 and EG.5.1 RBD display the mutation F456L that occur at the interface ACE2-RBD (Figure 1B), as already shown in XBB.1.15 and in other XBB sub-lineages (see https://gisaid.org/lineage-comparison/). The substitution of an aromatic ring with an aliphatic side chain may alter the interaction with ACE2 residues T27, D30, and K31. Indeed, prediction of the interaction energy between ACE2 and EG.5/EG.5.1 RBDs suggest a minor decrease in affinity with respect to XBB.1.5 (Supporting Information: Table S2) that differs from EG variants for the presence of Phe instead of Leu in position 456.

In conclusion, considering the genetic and structural data presented for SARS-CoV-2 EG.5, there is currently no evidence suggesting its heightened dangerousness or a probable high expansion capability. Although it has shown an increasing prevalence, as indicated by the WHO (https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—3-august-2023), it seems to exhibit lower virulence compared to other Omicron variants. Indeed, given that symptoms and the overall severity of the disease are currently less pronounced, an increase in prevalence is to be expected, but this does not pose a significant global threat. Any potential new features that could theoretically confer advantages are probably a result of genetic drift, enabling the virus to continuously adapt to its host. However, these changes may not necessarily be directly linked to increased contagiousness. Indeed, from an evolutionary point of view the key to survival and evolution lies not in strength or intelligence, but in adaptability to change. DNA and RNA have always been subject to changes, and viruses, being biological entities capable of employing complex strategies, host adaptation, and survival tactics, epitomize this adaptability (see i.a. Focosi et al.[12]). SARS-CoV-2 during the COVID-19 pandemic has proven to be a prime example of these biological features, undergoing significant mutations throughout the pandemic, leading to the emergence of multiple lineages and

sub-lineages exhibiting varying expansion capabilities.[8] The SARS-CoV-2 EG.5 variant represents the most recent outcomes of this phenomenon.

Nevertheless, this should not be taken as a reason to let our guard down against SARS-CoV-2, and the genome-based monitoring must continue uninterrupted to stay prepared and well-informed about the real situation.

AUTHOR CONTRIBUTIONS

Conceptualization: Fabio Scarpa and Massimo Ciccozzi. *Data analyses*: Fabio Scarpa, Stefano Pascarella, and Miriana Quaranta. *Writing-original draft preparation*: Fabio Scarpa and Stefano Pascarella. *Writing-review and editing*: Fabio Scarpa, Stefano Pascarella, Alessandra Ciccozzi, Marta Giovanetti, Ilenia Azzena, Chiara Locci, Marco Casu, Pier Luigi Fiori, Miriana Quaranta, Eleonora Cella, Daria Sanna, and Massimo Ciccozzi.

ACKNOWLEDGMENTS

This research was funded from by FONDAZIONE DI SARDEGNA bando 2022-2023 for the Dipartimento di Scienze Biomediche - UNISS (to Fabio Scarpa and Daria Sanna). Marta Giovanetti is funded by PON "Ricerca e Innovazione" 2014-2020. Marta Giovanetti is supported in part by the CRP-ICGEB RESEARCH GRANT 2020 Project CRP/BRA20-03, Contract CRP/20/03. Stefano Pascarella is in part supported by the Sapienza grant no. RP122181671E8B2F. We also would like to thank all the authors who have kindly deposited and shared genomes on GISAID.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the GISAID database at https://gisaid.org/. Genomes analyzed in the present study were taken from the GISAID database and are available at https://gisaid.org/.

ORCID

Fabio Scarpa http://orcid.org/0000-0002-3501-714X
Stefano Pascarella http://orcid.org/0000-0002-6822-4022
Marta Giovanetti http://orcid.org/0000-0002-5849-7326
Pier Luigi Fiori https://orcid.org/0000-0001-6190-612X
Massimo Ciccozzi https://orcid.org/0000-0003-3866-9239

REFERENCES

1. Borsetti A, Scarpa F, Maruotti A, et al. The unresolved question on COVID-19 virus origin: the three cards game? *J Med Virol*. 2022;94:1257-1260. doi:10.1002/jmv.27519
2. Kaku Y, Kosugi Y, Uriu K, et al. Antiviral efficacy of the SARS-CoV-2 XBB breakthrough infection sera against Omicron subvariants including EG.5. *bioRxiv*. Accessed August 8, 2023. doi:10.1101/2023.08.08.552415
3. Dyer O. Covid-19: infections climb globally as EG.5 variant gains ground. *BMJ*. 2023;382:1900. doi:10.1136/bmj.p1900
4. Scarpa F, Sanna D, Azzena I, et al. Genome-based comparison between the recombinant SARS-CoV-2 XBB and its parental lineages. *J Med Virol*. 2023;95(3):e28625. doi:10.1002/jmv.28625
5. Scarpa F, Giovanetti M, Azzena I, et al. Genome-based survey of the SARS-CoV-2 BF.7 variant from Asia. *J Med Virol*. 2023;95:e28714. doi:10.1002/jmv.28714
6. Mugosa B, Vujosevic D, Ciccozzi M, et al. Genetic diversity of the haemagglutinin (HA) of human influenza a (H1N1) virus in Montenegro: focus on its origin and evolution: genetic diversity of H1N1 in Montenegro. *J Med Virol*. 2016;88:1905-1913. doi:10.1002/jmv.24552
7. Scarpa F, Sanna D, Azzena I, et al. On the SARS-CoV-2 BA.2.75 variant: A genetic and structural point of view. *J Med Virol*. 2022;95:e28119. doi:10.1002/jmv.28119
8. Giovanetti M, Branda F, Cella E, et al. Epidemic history and evolution of an emerging threat of international concern, the severe acute respiratory syndrome coronavirus 2. *J Med Virol*. 2023;95(8):e29012.
9. Benvenuto D, Giovanetti M, Salemi M, et al. The global spread of 2019-nCoV: a molecular evolutionary analysis. *Pathog Glob Health*. 2020;114(2):64-67. doi:10.1080/20477724.2020.1725339
10. Pascarella S, Ciccozzi M, Bianchi M, Benvenuto D, Cauda R, Cassone A. The electrostatic potential of the Omicron variant spike is higher than in Delta and Delta-plus variants: a hint to higher transmissibility? *J Med Virol*. 2021;94(4):1277-1280. doi:10.1002/jmv.27528
11. Scarpa F, Imperia E, Azzena I, et al. Genetic and structural genome-based survey reveals the low potential for epidemiological expansion of the SARS-CoV-2 XBB.1.5 sublineage. *J Infect*. 2023;86:596-598. doi:10.1016/j.jinf.2023.02.039
12. Focosi D, McConnell S, Casadevall A. The omicron variant of concern: diversification and convergent evolution in spike protein, and escape from anti-spike monoclonal antibodies. *Drug Resist Updates*. 2022;65:100882. doi:10.1016/j.drup.2022.100882

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Scarpa F, Pascarella S, Ciccozzi A, et al. Genetic and structural analyses reveal the low potential of the SARS-CoV-2 EG.5 variant. *J Med Virol*. 2023;95:e29075. doi:10.1002/jmv.29075