

# **SARS-CoV-2 variant from India to Marseille: the still active role of ports in the introduction of epidemics**

Bernard LA SCOLA<sup>1,2\*</sup>, Philippe LAVRARD<sup>1,2</sup>, Pierre-Edouard FOURNIER<sup>1,2</sup>, Philippe COLSON<sup>1,2</sup>, Alexandre LACOSTE<sup>3</sup>, Didier RAOULT<sup>1,2</sup>

<sup>1</sup> Aix-Marseille Univ, IRD, APHM, MEPHI, Marseille, France

<sup>2</sup> Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France.

<sup>3</sup> Bataillon des marins pompiers de Marseille, Marseille, France

**\*Corresponding author:** Pr Bernard LA SCOLA

IHU Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille, France.

**Email:** [bernard.la-scola@univ-amu.fr](mailto:bernard.la-scola@univ-amu.fr)

A recent variant of SARS-CoV-2 named B.1.617 has spread to several countries from India [1]. The mutations found in the Indian variant in its spike are identified as E484Q, L452R, E154K and P681R. Amino acid 484 is changed in the South African variant B.1.351 and in the Brazilian variant P1 while L452R was already detected in a Californian variant. The association in a single variant of these mutations supposed to reduce recognition by antibodies and impact on attachment to the ACE2 receptor that has caused this strain to be classified as a variant of interest by the WHO due to a strong potential to cause epidemics [2,3].

We report herein the case of an Indian sailor coming to Marseille, south-east France, to embark as a crew member which illustrates the role of ports in the historical introduction of epidemics. This patient from Goa embarked at New Delhi airport, passed through Amsterdam airport and landed at Marseille airport on April 26, 2021. Tested SARS-CoV-2-negative 72

hours before boarding, he was detected positive upon arrival at Marseille by an antigen test. A new nasopharyngeal swab was performed for confirmation on April 27 and sent to our institute [4]. qRT-PCR was positive at Ct 17 and direct sequencing [5] confirmed the "Indian Variant" nature of this strain (Figure 1, Supplementary Figure S1). On April 28, characteristic CPEs were seen in culture (Supplementary Figure S2) and the strain sub-cultured for subsequent sero-neutralization analysis on the sera of local patients carrying antibodies (vaccinated and convalescent). So far, five B.1.617 cases were documented in France (<https://www.nouvelle-aquitaine.ars.sante.fr/communique-de-presse-coronavirus-point-de-situation-en-nouvelle-aquitaine-au-30-avril-2021>; <https://solidarites-sante.gouv.fr/actualites/presse/communiqués-de-presse/article/premieres-detections-de-cas-de-contamination-au-variant-b-1-617-du-sars-cov-2>).

This case perfectly illustrates the role played by ports such as Marseille in the entry of epidemics of distant origin. Indeed, for 2000 years this port has faced the arrival of epidemic agents, in particular plague, cholera, yellow fever. The history of these epidemics and the strategies put in place to fight them, including creation of our institute, have been recently reviewed [6]. For many years, merchant navy crews have mainly come from countries with low labor costs, in particular the Indian subcontinent, and the case of this sailor continues to illustrate this historical characteristic by the fact that it is an area of mixing of populations. It also raises the question of the lack of real control over transfers of people from areas where variants of concern are circulating. This patient had been tested before boarding and was able to transit unchecked to Marseille where, fortunately, civil security checks as many travelers as possible but without being exhaustive. It is very probable that similar situations will occur, illustrating the extreme difficulty of controlling the introduction of new epidemic variants in regions which are traditionally areas of intense transit. Ensuring effective detection of these cases is however critical, especially for crew members destined, as was the case with this

sailor, to embark on cruise ships in order to avoid a repetition of the Diamond Princess episode [7].

### **Acknowledgments**

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### **Ethics**

Data have been generated as part of the routine work at Assistance Publique-Hôpitaux de Marseille (Marseille university hospitals). This study has been approved by the ethics committee of our institution (N°2020-016-03) and written informed consent was obtained from the patient.

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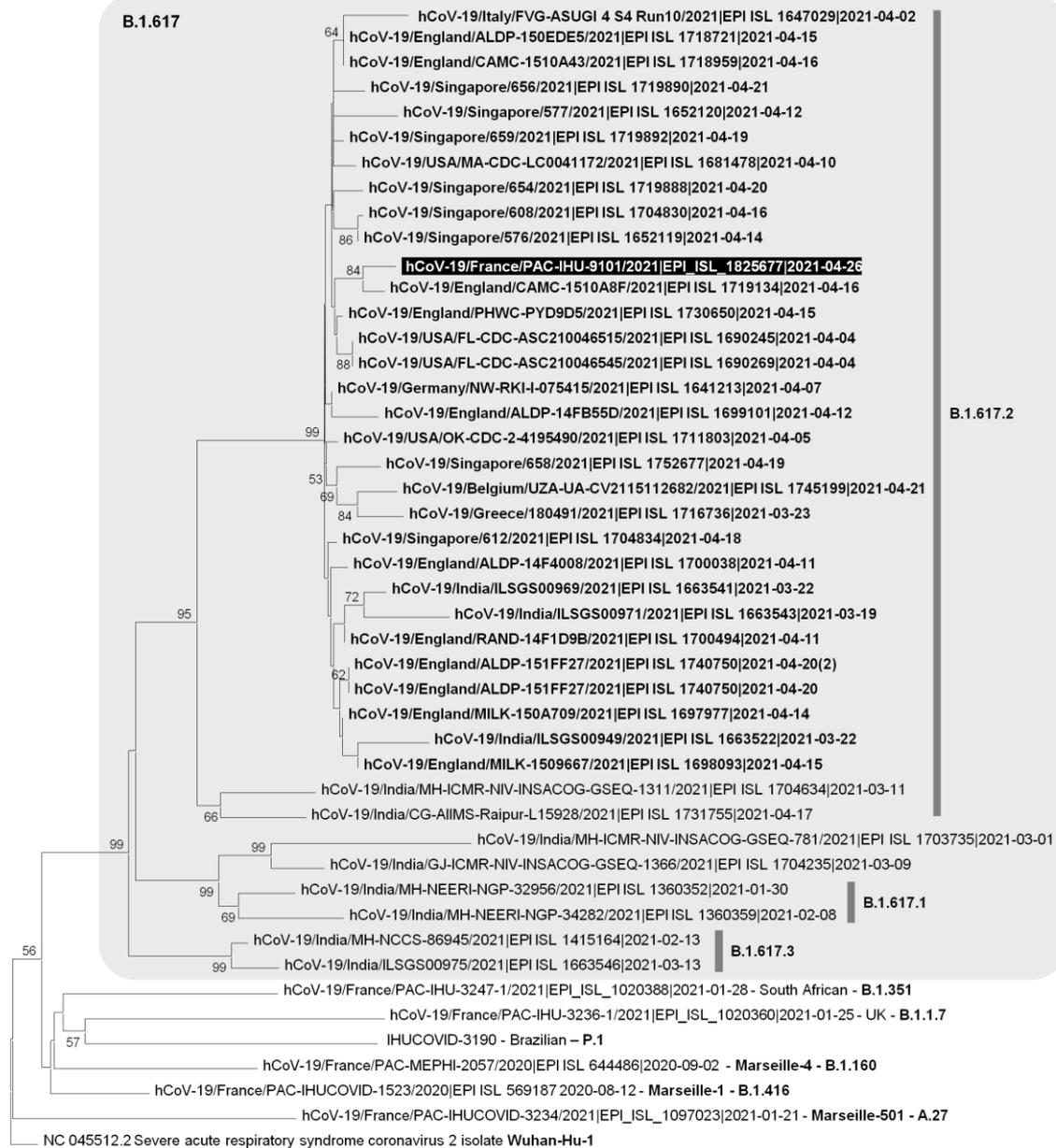
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**Figure 1.** Phylogeny reconstruction based on the SARS-CoV-2 full-length genomes.

The Marseille-IHU-9101 genome, indicated by a white bold font and a black background, is classified in Nextstrain clade 20A and Pangolin lineage B.1.617.2. It has been deposited in the GISAID database (<https://www.gisaid.org/>) [8] (No. EPI\_ISL\_1825677). Its best BLASTn hits (n= 30 selected) in GISAID have been obtained from samples collected recently (not earlier than the 19<sup>th</sup> of March 2021) in India, Singapore, the USA, and Western Europe (England, Italy, Belgium, Germany, Greece). This genome is strongly clustered (bootstrap value, 100%) with these most similar sequences in a subcluster of lineage B.1.617.2, and is clustered (bootstrap value, 88%) with one sequence obtained from a sample collected in England the same day than the present case-patient.

The SARS-CoV-2 phylogenetic tree is based on the full-length SARS-CoV-2 genomes. The 30 sequences with the highest BLAST scores recovered from the GISAID database (<https://www.gisaid.org/>) [8] were incorporated in the phylogeny reconstruction, being indicated by a black bold font. Additional sequences included the genome of the Wuhan-Hu-1 isolate and genomes obtained in our institute and classified as predominant variants.

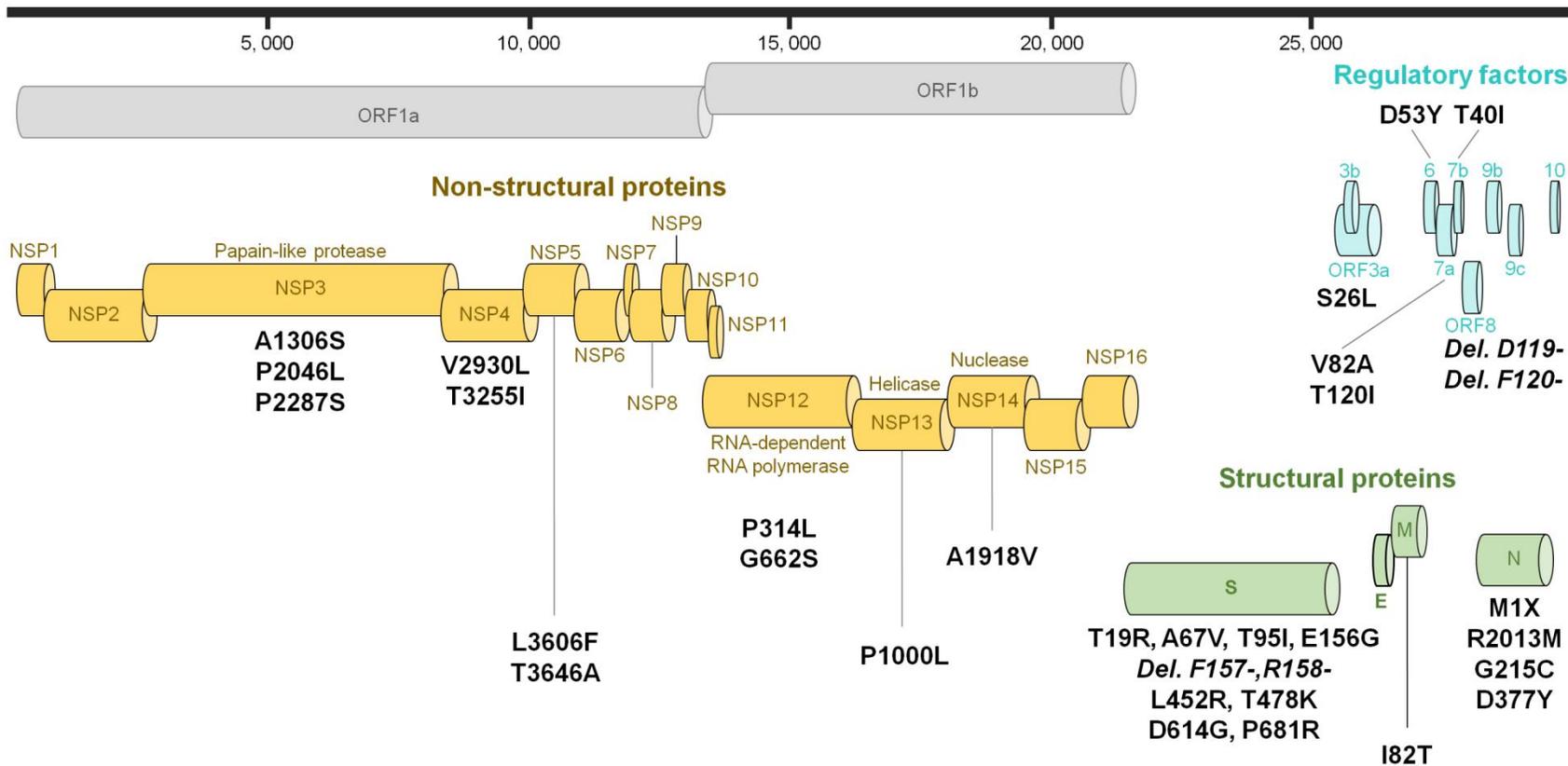
Nucleotide alignments were performed using the MUSCLE software (<http://www.ebi.ac.uk/Tools/msa/muscle/>). Evolutionary history was inferred using the MEGAX software (<http://www.megasoftware.net/>) using the neighbor-joining method and the Kimura 2-parameter method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree; the scale bars indicate the number of nucleotide substitutions per site. Bootstrap values > 50% are indicated on the tree. Pangolin lineages determined using the Pangolin COVID-19 Lineage Assigner (<https://pangolin.cog-uk.io/>) are indicated.



**Supplementary Figure S1.** Map of the amino acid changes along the SARS-CoV-2 genome.

The genome contains 36 nucleotide substitutions and 13 nucleotide deletions. They correspond to 29 amino acid substitutions and 4 amino acid deletions. Eight amino acid substitutions and 2 amino acid deletions are located in the spike protein, all in its S1 domain. Four substitutions (T19R, A67V, T95I, E156G) and the two deletions (F157-, R158-) are in the N-terminal domain, two substitutions (L452R, T478K) are in the receptor binding motif. Five amino acid substitutions are located in regulatory factors and two amino acid (D119-, F120-) are deleted from ORF8-encoded protein. Five substitutions are in the structural proteins. Other mutations include 3 in NSP3 (protease), 2 in NSP12 (RNA-dependent RNA polymerase), 1 in NSP13 (helicase) and 1 in nuclease (NSP14).

### SARS-CoV-2 genome



**Supplementary Figure S2.** Indian variant isolated in Marseille at IHU Méditerranée Infection. Cytopathic effect observed on Vero E6 cells after 24 hours of incubation.

