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Massive dissemination of a SARS-CoV-2 Spike Y839 variant in Portugal

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Abstract

Genomic surveillance of SARS-CoV-2 was rapidly implemented in Portugal by the National Institute of Health in collaboration with a nationwide consortium of >50 hospitals/laboratories. Here, we track the geotemporal spread of a SARS-CoV-2 variant with a mutation (D839Y) in a potential host-interacting region involving the Spike fusion peptide, which is a target motif of anti-viral drugs that plays a key role in SARS-CoV-2 infectivity. The Spike Y839 variant was most likely imported from Italy in mid-late February and massively disseminated in Portugal during the early epidemic, becoming prevalent in the Northern and Central regions of Portugal where it represented 22% and 59% of the sampled genomes, respectively, by April 30th. Based on our high sequencing sampling during the early epidemics [15.5% (1275/8251) and 6.0% (1500/24987) of all confirmed cases until the end of March and April, respectively], we estimate that, between March 14th and April 9th (covering the epidemic exponential phase) the relative frequency of the Spike Y839 variant increased at a rate of 12.1% (6.1%–18.2%, CI 95%) every three days, being potentially associated with 24.8% (20.8-29.7%, CI 95%; 3177-4542 cases, CI 95%) of all COVID-19 cases in Portugal during this period. Our data supports population/epidemiological (founder) effects contributing to the Y839 variant superspread. The potential existence of selective advantage is also discussed, although experimental validation is required. Despite huge differences in genome sampling worldwide, SARS-CoV-2 Spike D839Y has been detected in 13 countries in four continents, supporting the need for close surveillance and functional assays of Spike variants.

Keywords: SARS-CoV-2; COVID-19; genomic epidemiology; mutation; genetic variant; Spike; fusion peptide; D839Y

Introduction

The causative agent of COVID-19, the novel coronavirus SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), is a tremendous global threat, already leading to nearly 25 million confirmed cases and approximately 800 thousand deaths worldwide, as of August 30th, 2020 [1]. The Spike protein governs the binding of SARS-CoV-2 with its receptor angiotensin-converting enzyme 2 (ACE2) in human cells, the fusion between the viral and host cell membranes and, thus, the virus entry [2–8]. This protein, which decorates the virion surface, also induces neutralizing antibodies and is therefore the key target for vaccine development

[2,5,9]. In this context, it is of upmost importance to track the genetic diversity and evolution of circulating SARS-CoV-2 at regional and global levels, with special focus on detecting the emergence and monitoring the spread of Spike variants. Although surveillance has been expectedly focused on genetic changes affecting the Spike receptor binding domain (RBD) [2,10–12], changes in other domains should also be closely surveyed, particularly when they are linked to variant frequency increase at local, regional or global levels [13,14]. A variant carrying the Spike D614G mutation stands out as a remarkable example, as it became dominant worldwide during the first months of the pandemic [13,15,16] with recent studies suggesting that the G614 variant might be linked to an increased transmissibility but not pathogenicity [13]. Spike amino acid 614 is pocketed adjacent to the fusion peptide, which is the functional fusogenic element of the Spike protein [2], and near the expected cleavage site, suggesting that G614 might have induced a conformational change influencing the dynamics of the spatially proximal fusion peptide, thereby resulting in the altered infectivity [13]. Hence, other mutations of interest outside the RBD have been highlighted [13], particularly those falling within the Spike fusion peptide or proximal regions [2,13], due to the critical role of these motifs in inserting SARS-CoV-2 into the membrane of human cells [2,17]. The fusion mechanism is also pointed out as an important target for the development of specific drugs against coronavirus, since it is expectedly less mutable than the surface-exposed and immunogenic RBD [2,18]. The Spike amino acid 839, within the fusion peptide or proximal regions (there is still no consensus on their precise location) [2], is being highlighted due to its potential specific host-interacting role in Spike cleavage for SARS-CoV-2 fusion activation and/or in the induction of host inflammatory responses [13,19,20].

Here, we evaluated the temporal and geographical spread of a SARS-CoV-2 variant carrying the Spike protein amino acid change D839Y that had a massive dissemination during the early COVID-19 epidemic in Portugal after its introduction from Italy in mid-late February. After the globally dispersed Spike D614G mutation, this is the first study reporting the superspread of a Spike variant with a tremendous epidemiological impact at country level.

Material and methods

A detailed description of methods is available as Supplementary material.

Sample characterization

Samples used in this study were collected as part of the ongoing national SARS-CoV-2 laboratory surveillance conducted by the National Institute of Health (INSA) Doutor Ricardo Jorge, Portugal. SARS-CoV-2 positive samples (either clinical specimens or RNA) were provided by a nationwide network with >50 laboratories that was established at the beginning of the epidemic in Portugal. Available demographic information, date of sample collection, date of illness onset and travel history were provided by laboratories and Regional and National Health Authorities. Geographical data presented in this study refers to the Region (“Health Administration

region”), District or Municipality of the patients’ residence or, when no information is available (for a small proportion of cases), to the location of exposure or of the hospital/laboratory that collected/sent the sample.

SARS-CoV-2 genome sequencing and analysis

After cDNA synthesis, SARS-CoV-2 positive RNA samples were subjected to amplicon-based whole-genome amplification with tiled, multiplexed primers [21], following the ARTIC Consortium protocol (<https://artic.network/ncov-2019>; <https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuik6w>). After Illumina NexteraXT library preparation, paired-end sequencing was performed either on Illumina MiSeq or NextSeq 550, targeting ~1M reads per sample.

Analysis of sequence read data was conducted using the bioinformatics pipeline implemented in INSaFLU (<https://insaflu.insa.pt/>; <https://github.com/INSAFLU>), which is a web-based (and also locally installable) platform for amplicon-based next-generation sequencing data analysis [22].

Phylogenetic analysis and real-time data sharing on SARS-CoV-2 genetic diversity and geotemporal spread in Portugal

A total of 1516 SARS-CoV-2 genome sequences were analyzed in this study (corresponding to INSA’s collection as of July 23rd, 2020; Table S1) using the SARS-CoV-2 Nextstrain pipeline [23] version from March 23, 2020 (<https://github.com/nextstrain/ncov>).

A website (<https://insaflu.insa.pt/covid19>) was launched on March 28, 2020 for real-time data sharing on SARS-CoV-2 genetic diversity and geotemporal spread in Portugal, giving access to “situation reports” of the study and providing interactive data navigation using both Nextstrain (<https://nextstrain.org/>) [23] and Microreact (<https://microreact.org/>) [24] tools. To explore the frequency of Spike D839Y variant at worldwide level, we downloaded all the amino acid sequences (and associated metadata) of SARS-CoV-2 spike protein available at GISAID (as of 23 July 2020). The genome sequences with the D839Y mutation detected abroad were downloaded from GISAID (Table S2) and subjected to clade classification and integration into “global” and Portugal phylogeny using Nextstrain (<https://nextstrain.org/ncov>) and Nextclade (<https://clades.nextstrain.org/>).

Statistical analysis

In order to assess the temporal variation in the proportion of D839Y mutation among sequenced samples, a binomial regression model with logarithmic link function was applied. The model was then applied to extrapolate the evolution of Y839 cases in the total case population (data presented in Figure 4). To increase the robustness of the analysis, the studied timeframe (from March 14th to April 9th, overlapping the exponential phase of the epidemic in Portugal) was adjusted to ensure 3-days bins with at least 25 genome sequences. We assumed one day as the timeframe delay between sample collection and case notification. The Kruskal-Wallis

non-parametric test was used to assess the existence of statistically significant differences in Ct values between groups (Figure S4). Differences in Ct values for each pair of groups were assessed using the Wilcoxon test adjusted for multiple comparison tests.

Data availability

SARS-CoV-2 genome sequences generated in this study were uploaded to GISAID database (<https://www.gisaid.org/>). Accession numbers can be found in Supplementary material (Table S1).

Results

Introduction and spread of the SARS-CoV-2 Spike Y839 variant in Portugal

Acting as the National Reference Laboratory for SARS-CoV-2, INSA rapidly established the genome-based molecular surveillance of SARS-CoV-2 in Portugal. A website (<https://insaflu.insa.pt/covid19>) was launched, providing updated data regarding the analysis of the SARS-CoV-2 genetic diversity and geotemporal spread. As of July 23, 2020, INSA had analysed 1516 genome sequences (Table S1), enrolling 15.5% (1275/8251) and 6.0% (1500/24987) of all confirmed cases detected until the end of March and April, respectively. According to Nextstrain clade definition (<https://clades.nextstrain.org/>), the 1516 SARS-CoV2 genomes from Portugal (<https://microreact.org/project/nDGsJKFv7gQTj1q8CQwwKR/18a0a470>) follow, in general, the same trend observed at European level (<https://nextstrain.org/ncov/europe>) [15,25]. Most viruses (89.8%) integrate the phylogenetic branch enrolling clades 20A (40.8%), 20B (46.1%) and 20C (2.9%), carrying, among other genetic markers, the D614G amino acid replacement in the Spike protein [13,15,16]. Clades 19A and 19B were found at the relative frequencies of 7.4% and 2.8%, respectively.

Within clade 20A, a SARS-CoV-2 variant carrying the Spike amino acid change D839Y (due to a G24077T SNP) was detected early (March 7th, 2020) during the COVID-19 epidemic in Portugal. The Spike Y839 variant was most likely imported from Italy in mid-late February 2020, as first detected genomes were all collected from individuals from the Northern region of Portugal that contacted (primarily or secondarily) with epidemiologically linked infected individuals that had been in Milan for an international trade fair during the third week of February 2020. Concordantly, the first D839Y genome sequence reported worldwide was collected in Italy (Lombardy) on February 21st (Italy/PV-5314-N/2020; GISAID accession number EPI_ISL_451307) (Table S2) [26], being identical (i.e, Nextstrain clade 20A background plus the G24077T SNP) to the “founder” Spike Y839 variant genome sequences detected in Portugal (<https://insaflu.insa.pt/covid19>). So far, current data and ongoing epidemiological investigations support a single origin (Milan, Italy) and, although there was a substantial representation of Portuguese companies in this event, available data links the introduction of the Y839 variant in Portugal to a single industrial area in the North of Portugal. The Spike Y839 variant became particularly prevalent in Portugal, representing about 20% of all sampled genomes collected until the end of

March (255/1275) or the end of April (287/1500) (Figure 1). Its circulation was particularly marked in Northern (epicenter of COVID-19 epidemic in Portugal) and Central regions of Portugal, representing 22% and 59% of the sampled genomes, respectively, by the end of April 2020 (Figures 2 and 3; <https://micrreact.org/project/nDGsJKFv7gQTj1q8CQwwKR/0489f840>). In the same period, four districts (with at least 50 genomes sampled as of April 30th) revealed the highest Y839 relative frequency: Braga (16.3%), Porto (25.2%), Aveiro (68.1%) and Guarda (72.7%) (Figure S1). We estimate that the relative frequency of Y839 increased at a rate of 12.1% (6.1%–18.2%, CI 95%) every three days between March 14th and April 9th, increasing from 13.3% to 33.1% (Figure 4). It was potentially associated with 3793 (3177–4542, CI 95%) COVID-19 cases in Portugal during that period, representing 24.8% (20.8–29.7%, CI 95%) of the total confirmed cases reported in the same period (Figure 4). Hence, our data supports that the Spike Y839 variant was circulating in Portugal since mid-late February (more than one week before the first COVID-19 confirmed case at March 2nd, 2020), being most likely responsible for the largest SARS-CoV-2 transmission chain occurred during the first 1–1.5 months of COVID-19 epidemic in Portugal. In particular, the Spike Y839 variant is strongly linked to a large and worrying COVID-19 “local” outbreak occurring in a small municipality (Ovar) in the coastal side of the Central region of the country (District of Aveiro), with 80% of the genomes collected from this municipality carrying the Y839 variant. Ovar was the only municipality in Portugal mainland that was subjected to strict local quarantine and lockdown measures (from March 17th to April 17th), presenting an incidence of 636 cases per 100 000 inhabitants in the last 14 days by April 5th. The Public Health Unit of Primary Care Cluster of Baixo Vouga (covering several municipalities, including Ovar), has been carrying out a deep investigation leading to the identification of clusters of potential epidemiologically linked confirmed cases (“epiclusters”) among 1556 monitored COVID-19 cases (by April 30th). Our SARS-CoV-2 genome collection (as of April 30th) includes samples representative of 41 of those potential epiclusters (covering a total of 420 confirmed cases), of which 33 (323 confirmed cases, 77%) are exclusively associated with the Spike Y839 variant (Figure S2). In a conservative manner, it is reasonable to extrapolate that the Spike Y839 variant is potentially associated with about 1200 cases (77% of the total 1556 monitored cases) in the region covered by Primary Care Cluster of Baixo Vouga. Still, the Spike Y839 variant disseminated far beyond the coastal municipality of Ovar and neighborhood municipalities. In the inland region of the country, Y839 variant was for instance linked to a large cluster of infected individuals living/working in a nursing home in Vila Nova de Foz Côa municipality (District of Guarda) detected by the end of March, and more than 50% of Y839 genomes detected in April were collected in the District of Viseu (Figure S1; Figure S3; <https://micrreact.org/project/2kh3TRVYB9gWGRpNSJWDW5/b6c659e0>), which borders the district of Aveiro. In total, the highly prevalent Spike Y839 variant was already detected in 44 municipalities across 11 out of the 18 Districts of Portugal mainland, consolidating that this descendent variant of the globally spread G614 variant had a remarkable weight in the early COVID-19 epidemic in Portugal.

Detection and circulation of SARS-CoV-2 Spike 839 amino acid variants worldwide

To explore the frequency of SARS-CoV-2 D839Y mutation (and other mutations in 839 protein position) at worldwide level, we downloaded 66548 amino acid sequences (and associated metadata) of SARS-CoV-2 Spike protein available at GISAID (as of 23 July 2020). From the 65367 sequences collected outside Portugal having data for the 839 position, 97 were found to display mutations in this amino acid of interest (Table 1, Table S2). Of those, 92 genomes revealed the same G24077T nucleotide substitution (leading to the D839Y amino acid replacement) in a SARS-CoV-2 with Spike G614 background. As previously noticed¹³, this data sustains that the global frequency of amino acid variants in this 839 site remains around 0.5% (similar estimates can be found in <https://cov.lanl.gov/content/index> and <https://bigd.big.ac.cn/ncov/>). After the first Spike Y839 variant was detected in Italy (Lombardy) on February 21st, the mutation D839Y has been reported in 12 other countries from four continents (Europe, Oceania, Asia and America) (Figure 5). Despite the highly unequal sequencing “sampling” (i.e., proportion of confirmed cases with SARS-CoV-2 genome data) between the different countries, it is noteworthy that, apart from Portugal, D839Y genomes represent ~5% of all sequences made available at GISAID (as of July 23rd, 2020) by three countries (Estonia, Georgia and New Zealand) (Figure 5). Of note, the four genomes detected in Iceland on early March are also associated with travel history to Italy [15,27] (Table S2). Notwithstanding, fine-tune integration of the 92 Spike Y839 genomes detected abroad in the “global” phylogeny (using Nextstrain <https://nextstrain.org/ncov> and Nextclade <https://clades.nextstrain.org/>) pointed out that the D839Y amino acid change likely emerged independently in two other instances. This observation is supported by one genome sequence collected in the United Kingdom (Wales) on April 24th (Wales/PHWC-35B01/2020; GISAID accession EPI_ISL_474528), which presents all clade-defining SNPs of Nextstrain Clade 20C plus four additional SNPs (including G24077T). In this particular case, we cannot exclude the hypothesis that the G24077T nucleotide change might have been introduced in a clade “20C” SARS-CoV-2 by recombination, as “20C” and “20A harboring Y839” viruses co-circulated in Wales during the collection period (13 out of the 51 Spike 839Y genomes from UK were collected in Wales between April 10th and May 25th). The potential third independent emergence of a Spike Y839 variant is supported by the four genomes collected in India, as they cluster apart from other G614+Y839 genomes, forming a sub-branch (supported by 6 SNPs, including G24077T) within a large cluster mostly enrolling genomes from India.

Impact of Spike Y839 variant on viral load

The Cycle threshold (Ct) obtained in diagnostic PCR is an indirect indicator for relative viral loads *in vivo*, with lower Ct values indicating higher viral loads. Increasing reports have been linking the globally dispersed Spike G614 variant to lower Ct values [13,28,29], suggestive of higher upper respiratory tract viral loads leading to increased G614 transmissibility, but not pathogenicity [13]. INSA gathered Ct values of 940 out of the 1516 genomes analyzed in present study. Despite the expected bias associated with sample collection (e.g., momentum), sampling (e.g., selection) and testing (e.g., extraction, PCR protocol and equipment) within datasets enrolling multiple laboratories, we sought to verify whether the same trend is observed for the D614/G614 comparison and whether the Spike Y839 variant (Spike background G614+Y839) may also be

potentially linked with changes in Ct values. As previously seen [13,28,29], we observed that samples with Spike G614 had Ct values (mean=22.2; n=844) lower than those with D614 viruses (mean=22.9; n=96), although without statistical significance (Figure S4). Considering the phylogeny of SARS-CoV-2 in Portugal, we then compared Ct values according to D614/G614 status, phylogenetic group within G614 (i.e., 20B or non-20B) and D839/Y839 status (Figure S4; <https://microreact.org/project/nDGsJKFv7gQTj1q8CQwwKR/f46flfa4>). Curiously, 20B clade (defined by the GGA-to-AAC SNP triplet at genome position 28881-3) revealed the lowest Ct values (mean=21.7; n=413) among all groups, although without statistical significance in pairwise comparisons. Regarding the D839/Y839 comparison, we observed that Y839 presented non-significant higher Ct values (mean=22.7; n=220) than the ancestral D839 (mean=22.1; n=720). However, considering that D839 includes both 20B and non-20B samples, contrarily to Y839, which is a sub-group within 20A (Figure S4), we repeated the analysis by excluding 20B samples from the comparison and observed similar average Ct values in D839 (mean=22.6; n=624) and Y839 (mean=22.7; n=220) groups. When applying the same rationale (i.e., excluding 20B) to the D614/G614 comparison, the 0.7 difference observed in the mean Ct values using the whole dataset decreased to less than 0.3.

Discussion

One of the main objectives of conducting genome-based surveillance of circulating pathogens is to identify mutations potentially leading to fitness advantages and/or immunological/drug resistance. In this perspective, mutations in the SARS-CoV-2 Spike D839 site, which is predicted to fall within the Spike fusion peptide or proximal regions [2,13], are of particular interest given both its non-negligible relative frequency worldwide and the pivotal role of this protein region in inserting SARS-CoV-2 into human cell membranes [2,13]. In the present study, we show that a SARS-CoV-2 variant with a Spike D839Y mutation was associated with ~25% of all COVID-19 cases in Portugal during the exponential phase of the epidemic, after its importation from Italy in mid-late February 2020. Our data shows that it was circulating in Portugal before the first COVID-19 confirmed case was detected on March 2nd, becoming notably prevalent in the Northern (22%) and Central (59%) regions of the country by the end of April 2020 (Figures 2 and 3).

The massive dissemination of the Spike Y839 variant in Portugal might be due to a populational/epidemiological effect (founder effect). This variant was likely one of the first SARS-CoV-2 to be introduced in Portugal, so it might have had more opportunity to spread. Its introduction is strongly linked to an international trade fair in Milan with many Portuguese attendees. So, we can raise the possibility of several “simultaneous” introductions of Y839 variant in Portugal, although this scenario would likely imply its circulation in the South of Portugal and abroad, namely in Spain (also highly represented in the event) where it has not been detected so far (<http://seqcovid.csic.es/nextspain/>, as of August 30th) despite the high sequencing sampling. The alternative scenario of a single introduction is then plausible considering that travel history and contact tracing data collected to date links all initial cases to a single industrial area in the North of Portugal and does not indicate

additional introductions. Regardless of the scenario, the increasing frequency trajectory of Spike Y839 variant would not have been mitigated because this variant disseminated well before the first confirmed cases in Portugal, when contact tracing, broad testing, and strict lockdown measures were still not in place. For instance, on March 9th, case definition for a COVID-19 suspected case did not include individuals with acute respiratory infection, unless they required hospitalization, reported travel history or any contact with suspected or confirmed cases in the 14 days before symptoms onset (<https://www.dgs.pt/directrizes-da-dgs/orientacoes-e-circulares-informativas/orientacao-n-002a2020-de-25012020-atualizada-a-250220201.aspx>). In another perspective, one cannot rule out that the high dissemination could have also been driven by fitness increase mediated by the D839Y mutation, which would be consistent with its estimated frequency increase from 13.3% to 33.1% in a 4-week period. In this hypothesis, this mutation would have posed “advantageous” structural changes in the Spike protein with potential impact on SARS-CoV-2 infectivity and, consequently, on its transmissibility, as suggested for D614G [13]. While D614G was hypothesized to increase SARS-CoV-2 infectivity by influencing the dynamics of the spatially proximal fusion peptide [13], D839Y, which itself falls within functional fusogenic element of Spike[2,13], could also have shaped this motif towards a better fitted fusion of SARS-CoV-2 with human cells. Also, recent data based on computational modeling suggested that mutations in Spike D839 may strengthen the interaction between the virus and human T cells potentially influencing host inflammatory responses [in particular, when replacing the aspartic acid (D) to an aromatic tyrosine (Y)] [19], and may influence Spike cleavage by host proteases during SARS-CoV-2 fusion activation and entry [20]. Nevertheless, these clues about the potential impact of this mutation on SARS-CoV-2 transmissibility/pathogenicity require evidence through experimental validation to verify or rule out the fitness advantage hypothesis. Although we did not observe any association between Y839 and Ct values (viral load) in our dataset, this assessment needs to be revisited as more data is acquired and be complemented with other assays. Notwithstanding, we indirectly observed that samples from clade 20B had a lower mean Ct value than non-20B groups in our dataset, supporting that it is worth performing this comparison with additional datasets and that the evaluation of (sub)clade effects should not be neglected in this kind of screenings.

Besides the potential functional role of D839Y mutation and its high prevalence in Portugal, its detection in 12 other countries from four continents, its probable independent emergence in distinct times and genetic clades (20A and 20C) in some of these countries and its considerable relative weight (~5%) in the sampled genomes of three countries (besides Portugal) also indicate that the hypothesis of selective advantage is not implausible. Contrarily to Spike G614 variant, which emerged way before the general quarantine in Europe, the Spike Y839 variant likely emerged on mid-late February in Lombardy, Italy. This is strongly corroborated by the detection of both D839 and Y839 subpopulations in different anatomical sites of the Y839-infected individual in Lombardy, Italy, by February 21st [26]. At this time, rigid lockdown measures started being implemented everywhere in Europe, which posed strong bottlenecks on SARS-CoV-2 population, likely giving Y839 variant less opportunity to expand, even if it was selectively advantageous over other circulating variants. Nonetheless, in Portugal, contrarily to what might have happened in other countries, the timeline of the epidemics certainly

avored a high weight of “founder effect” in the remarkable dissemination of the Y839 variant. For instance, first lockdowns in Lombardy, Italy, where Spike Y839 variant was firstly detected on February 21st, coincidentally began on this date [30]. In contrast, in Portugal, first cases were confirmed in March 2nd and national quarantine was implemented on March 18th, when Spike Y839 variant had already been circulating in the community for at least three weeks. It is still worth highlighting that the huge discrepancies in sequencing sampling between countries completely hampers a real knowledge of D839Y frequency regionally and globally. Our sequencing sampling after April 30th does not allow us to infer the current relative frequency of the Spike Y839 variant in Portugal. One can speculate that its circulation was highly contained considering that, after this period, the epidemic evolved favorably in Northern and Central regions (where Y839 had mostly circulated), contrarily to the Lisbon and Tagus Valley region (where Y839 was, at that time, rarely seen) (http://www.insa.min-saude.pt/wp-content/uploads/2020/08/Report_covid19_07_08_2020.pdf).

In summary, we describe the emergence and increase in frequency of a Spike Y839 variant that reached a tremendous impact on COVID-19 epidemic in Portugal, as estimated by its high relative weight of one in each four cases during the exponential phase of the epidemic. Similarly to other major Spike variants, Y839 certainly constitutes an important target for functional and immunological studies. Our study reinforces the need for continuous and close surveillance of SARS-CoV-2 genetic diversity, with emphasis on detecting and monitoring variants with potential impact at biological and/or epidemiological levels.

Ethical Approval

This study was approved by the Ethical Committee (“Comissão de Ética para a Saúde”) of the Portuguese National Institute of Health.

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Disclosure statement

No conflicts of interest were declared.

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Table 1. Overview of the SARS-CoV-2 Spike amino acid sequences with mutations in the 839 site available at GISAID, as of July 23rd, 2020.

Spike mutation	D614G background	count	countries ^{a, b}	date of collection range ^b
D839Y	G614	382	Italy (1), United Kingdom (51), Iceland (4), Portugal (290) , Georgia (1), Poland (1), Netherlands (9), New Zealand (14), Switzerland (4), Austria (1), USA (1), Estonia (1), India (4)	21/Feb - 17/Jun
D839E	G614	1	Netherlands (1)	28/Feb
D839N	G614	1	Australia (1)	20/Jun
D839N	D614	1	United Kingdom (1)	25/Mar
D839G	D614	2	United Kingdom (2)	24/Mar - 08/May

^aCountries are ordered by the date of collection of the first reported genome with the Spike 839 site variant. Individual sequences are detailed in Table S1 (Portugal) and S2 (abroad).

^bThe reported case in Estonia has March 2020 as the date of collection (March 31st 2020 was assumed in the Figure 5). Two genomes (one from United Kingdom and another from India) only had the year of sampling available. These were not included in Figure 5.

Figures legends

Figure 1. Overview of the SARS-CoV-2 genome sequencing sampling in Portugal and cumulative relative frequency of the circulating Spike Y839 variant, as of April 30th, 2020 (n=1500). Area plots (left y-axis) reflect the cumulative total number of SARS-CoV-2 genome sequences (gray) and Spike Y839 variant sequences (red) obtained in Portugal during the first two months of the epidemic. Lines (right y-axis) display the cumulative percentage of COVID-19 confirmed cases for which SARS-CoV-2 genome data was generated (“sequencing sampling” - black dash line) and the cumulative proportion of the Spike Y839 variant sequences (red line) detected in Portugal during the same period.

Figure 2. Landscape of the geotemporal spread of SARS-CoV-2 Spike Y839 variant in Portugal by region, as of April 30th, 2020. **A.** Distribution of the analysed genome sequences (n=1500) by date of sample collection and Health Administration region, highlighting COVID-19 cases caused by the Spike Y839 variant (red dots). In y-axis, it is indicated, for each region, the number of sequences analysed (n), the percentage of confirmed cases with SARS-CoV-2 genome data (% cases), the percentage of sequences from each region in the whole dataset (% genomes) and the percentage of Y839 variant sequences (%Y839, in red), as of April 30th, 2020. **B.** Radial maximum likelihood phylogenetic tree showing the high proportion of genomes with the Spike D839Y mutation detected in Portugal [about 20% of all sequences collected until the end of March (255/1275) or the end of April (287/1500)]. This dataset covers 15.5% (1275/8251) and 6.0% (1500/24987) of all confirmed cases detected until the end of March and April, respectively. The phylogeny and geotemporal distribution can be visualized interactively at <https://microreact.org/project/nDGsJKFv7gQTj1q8CQwwKR/0489f840> (geographic resolution by Region) and <https://microreact.org/project/2kh3TRVYB9gWGRpNSJWDW5/b6c659e0> (geographic resolution by District) using Microreact (<https://microreact.org/>). **C.** Distribution of the Spike Y839 variant by Health Administration region, highlighting its high relative frequency in the Northern and Central regions of Portugal, where this variant represented 22% and 59% of the sampled genomes until the end of April 2020, respectively. The size of the pie charts is proportional to the number of sequenced genomes. **D.** Cumulative total number of COVID-19 confirmed cases by Health Administration region, showing the Northern region as the “epicenter” of the epidemic during the two first months (source: General Directorate of Health (DGS), <https://covid19.min-saude.pt/relatorio-de-situacao/>).

Figure 3. Overview of the SARS-CoV-2 genome sequencing sampling and cumulative relative frequency of the circulating Spike Y839 variant, as of April 30th, 2020 (n=1500), in the Northern (A) and Central (B) regions of Portugal. Area plots (left y-axis) reflect the cumulative total number of COVID-19 confirmed cases (light blue) and SARS-CoV-2 genome sequences (dark blue) detected/generated in each Health Administration region. Lines (right y-axis) display the cumulative percentage of COVID-19 confirmed cases with SARS-CoV-2 genome data, i.e., sequencing sampling (blue dash line) and the cumulative proportion of the Spike Y839 variant sequences (red line) detected in those regions during the same period.

Figure 4. Increasing trajectory of the Spike Y839 variant and estimated weight of this variant in the total number of COVID-19 confirmed cases in early epidemic. A binomial regression model with logarithmic link function was applied to assess the temporal variation in the proportion of the Y839 variant among sequenced samples (graph in the upper left corner, showing an estimate increase from 13.3% to 33.1%). This model was then applied to extrapolate the evolution of Y839 cases (red line) in the total confirmed case population (gray bars) at each 3-day interval (main graph). Crosses represent the estimated Y839 cases and the shaded region shows the 95% confidence interval. 1-day was assumed as the timeframe delay between sample collection and case notification.

Figure 5. Detection and circulation of the SARS-CoV-2 Spike D839Y mutation worldwide. SARS-CoV-2 Spike amino acid sequences available at GISAID (<https://www.gisaid.org/>), as of July 23rd, 2020, were download, aligned and screened for the presence of mutations in Spike 839 amino acid position. The main plot display the country and data of collection of 92 Spike sequences with the D839Y mutation (detailed in Table S2). The bar graph in the upper right corner displays the proportion of D839Y sequences in the total number of Spike amino acid sequences available per country. As detailed in Table S2, the D839Y sequence from Estonia indicates March 2020 as the date of collection (March 31st 2020 was assumed in this plot). Two genomes (one from United Kingdom and another from India) only had the year of sampling available, thus they were not included in the graph.

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Supplementary Information

Supplementary methods

SARS-CoV-2 amplicon-based genome amplification and sequencing

SARS-CoV-2 positive RNA samples were subjected to genome sequencing using a whole-genome amplification strategy with tiled, multiplexed primers¹ and the Artic Consortium protocol (<https://artic.network/ncov-2019>; <https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuk6w>), with slight modifications. In brief, after cDNA synthesis using SuperScript™ IV First-Strand Synthesis System kit (Invitrogen™, catalog: 18091050) with random hexamers from 11 µL of RNA (exactly as described in Artic Network protocol), targeted amplification was performed with 2.5 µL of cDNA using NEBNext® Q5® HotStart HiFi Master Mix (12.5 µL per reaction) (New England BioLabs, catalog: M0544S) with two pools of tiling primers (A and B) separately. Primers versions V1 and V2 (aliquots kindly provided by Artic Network team) were used for the first 243 samples of this study, while the V3 primers (with a total of 218 primers) were applied to all samples afterwards (all versions available here: (https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019)). The final concentration per primer (V3 version) was ~0.013µM (1.4 µM per pool) in a 25 µL total reaction volume. PCR amplification parameters were: 30s at 98°C, 35 cycles of 15s at 98°C and 5 min at 65°C (for the first 762 samples) or 63°C (afterwards), and final extension for 5 min at 65/63°C. Amplicons were visualized on a 1% agarose gel, tubes A and B were pooled per sample, and subjected to clean up with Agencourt AMPure XP (Beckman Coulter, catalog: A63880) using a 1:1 volume ratio. Purified amplicons were quantified using Qubit fluorometer (Thermo Fisher Scientific) and normalized to a concentration of 0.4 ng/ul. Dual-indexed sequencing libraries were prepared using Illumina Nextera XT DNA Library Prep Kit (Illumina). Pooling, denaturation and dilution of bead-based normalized libraries was performed according to the manufacturer's instructions for the MiSeq or NextSeq 550 systems (Illumina). A 1% spike-in PhiX genome library (Illumina) was used as internal quality control. Libraries were sequenced using 250bp (MiSeq) or 150bp (NextSeq 550) paired-end reads targeting ~1M reads per sample.

Genome assembly and sequence curation

Analysis of sequence read data was conducted using the bioinformatics pipeline implemented in INSaFLU (<https://insaflu.insa.pt/>; <https://github.com/INSaFLU>), which is a web-based (and also locally installable) platform for amplicon-based next-generation sequencing data analysis². Briefly, the core bioinformatics steps (documented in Borges et al, 2018 and <https://insaflu.readthedocs.io/>) involved: i) raw NGS reads quality analysis and improvement using FastQC; (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc>) and Trimmomatic (<http://www.usadellab.org/cms/index.php?page=trimmomatic>), respectively (read's ends were cropped 30bp for primer clipping); ii) draft *de novo* assembly using SPAdes (<http://cab.spbu.ru/software/spades/>) followed by classification and contigs assignment of Human Betacoronavirus; and, iii) reference-based mapping, consensus generation and variant detection using the multisoftware tool Snippy (<https://github.com/tseemann/snippy>), using the Wuhan-Hu-1/2019 genome sequence (<https://www.ncbi.nlm.nih.gov/nuccore/MN908947>) as reference. The first 40bp and end 100bp were discarded

and consensus sequences were exclusively included in the study when >70% of genome was covered by at least 10-fold. For samples with coverage drop below 10-fold, fine-tuned consensus sequence curation was performed as follows: i) undefined bases ("N") were placed in genome regions with depth of coverage below 10 using a python script (https://github.com/rfm-targa/BioinfUtils/blob/master/msa_masker.py), and "N" regions at the sequence ends were trimmed to avoid releasing sequences starting or ending with "N"; ii) all regions with depth coverage below 10 were visually inspected in the Integrative Genomics Viewer (<http://www.broadinstitute.org/igv>) available *in situ* at INSAFLU, and when mutations were detected and validated within these low coverage regions, they were inserted in the consensus sequence to avoid disrupting/biasing the phylogenetic signal; iii) when a mutation was validated within a "N" region with ≤ 100 bp, the whole region was inserted (as long as the region was covered by at least one read); when a mutation was validated within a "N" region with > 100 bp, the mutation was inserted together with additional 20 bp (10bp from each flanking side) to improve the downstream sequence alignment.

SARS-CoV-2 Phylogenetic analysis using Nextstrain

A total of 1516 SARS-CoV-2 genome sequences were analyzed in this study (corresponding to INSA's collection as of July 23rd, 2020; Table S1) using the SARS-CoV-2 Nextstrain pipeline³ version from March 23, 2020 (<https://github.com/nextstrain/ncov>), with slight modifications. In brief, sequences were aligned against the reference Wuhan-Hu-1/2019 genome of SARS-CoV-2 (GenBank accession MN908947) using MAFFT⁴, the alignment was visually inspected, manually curated and further used to build a maximum likelihood phylogenetic tree based on the GTR model using IQ-TREE⁵ following the Nextstrain implementation (the first 130bp and last 50 bp, as well as a few bases within the alignment, were masked as likely sequencing artifacts). Within the Nextstrain pipeline, Treetime⁶ is applied to infer a time-resolved phylogeny. The phylogeny is rooted relative to early samples from Wuhan, China (Wuhan-Hu-1/2019, GenBank accession MN908947; Wuhan/WH01/2019, GenBank accession LR757998) and temporal resolution assumes a nucleotide substitution rate of 0.0008 ± 0.0004 substitutions *per site per year*.

Real-time data sharing of SARS-CoV-2 genetic diversity and geotemporal spread in Portugal

A website (<https://insaflu.insa.pt/covid19>) was launched on March 28, 2020 for real-time data sharing on SARS-CoV-2 genetic diversity and geotemporal spread in Portugal. This site gives access to "situation reports of the study and provides interactive data navigation using both Nextstrain (<https://nextstrain.org/>)³ and Microreact (<https://microreact.org/>)⁷ tools. As of July 23rd, 2020, "clade" assignment reflects the Nextstrain classification (<https://github.com/nextstrain/ncov>; version June 3, 2020), while "Lineage" refers to the classification based on Phylogenetic Assignment of Named Global Outbreak Lineages (Pangolin) (<https://github.com/hCoV-2019/pangolin> - lineage_version: 2020-05-07)⁸.

In this study, both Nextstrain (<https://nextstrain.org/>)³ and Microreact (<https://microreact.org/>)⁷ visualization tools were used to deeply explore the genetic diversity and geotemporal spread dynamics of SARS-CoV-2 Spike D839Y variant in Portugal. The phylogeny and associated metadata used in this study can be visualized interactively at <https://microreact.org/project/nDGsJKFv7gQTj1q8CQwwKR/18a0a470> (global dataset, as of July 23rd, 2020), <https://microreact.org/project/nDGsJKFv7gQTj1q8CQwwKR/0489f840> (geographic resolution by Region, genomes collected until April 30th highlighted) and

<https://microreact.org/project/2kh3TRVYB9gWGRpNSJWDW5/b6c659e0> (geographic resolution by District, genomes collected until April 30th highlighted)) using Microreact (<https://microreact.org/>)⁷. To explore the frequency of SARS-CoV-2 Spike D839Y variant at worldwide level, we downloaded 66548 amino acid sequences (and associated metadata) of SARS-CoV-2 spike protein available at GISAID (as of 23 July 2020) and aligned them using MAFFT⁴. A total of 65367 sequences collected outside Portugal had sequence data for amino acid position 839, giving a total of 66883 (65367 plus 1516) sequences screened in this study for the presence of mutations in this amino acid of interest. The 92 genomes sequences of the SARS-CoV-2 Spike D839Y variant detected abroad were downloaded from GISAID (GISAID acknowledgments are in Table S2). Their clade classification and integration into “global” and Portugal phylogeny was performed using Nextstrain (<https://nextstrain.org/ncov>) and Nextclade (<https://clades.nextstrain.org/>).

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Supplementary Figures and Tables

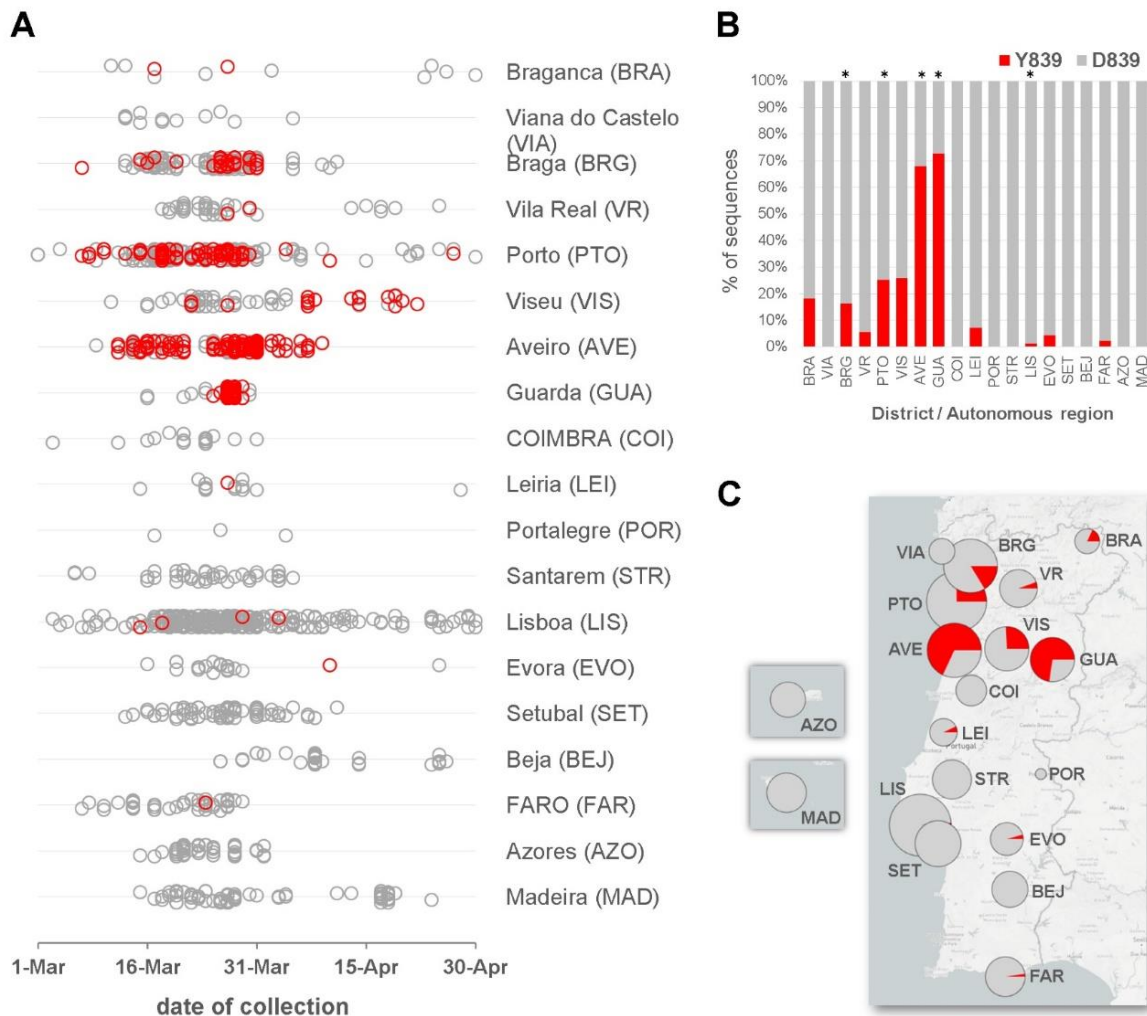


Figure S1. Landscape of the geotemporal spread of SARS-CoV-2 Spike Y839 variant in Portugal by District, as of April 30th, 2020. **A.** Distribution of the analysed genome sequences (n=1500) by date of sample collection and District, highlighting COVID-19 cases caused by the Spike Y839 variant (red dots). **B.** Relative frequency of the Spike Y839 variant across the 11 Districts where the variant was detected until April 30th. Asterisks above the graph denote Districts where more than 50 genomes were sampled. **C.** Relative frequency of Spike Y839 variant by District until the end of April 2020. The phylogeny and geotemporal distribution can be visualized interactively at <https://microreact.org/project/nDGsJKFv7qQTj1q8CQwwKR/0489f840> (geographic resolution by Region) and <https://microreact.org/project/2kh3TRVYB9gWGRpNSJWDW5/b6c659e0> (geographic resolution by District) using Microreact (<https://microreact.org/>).

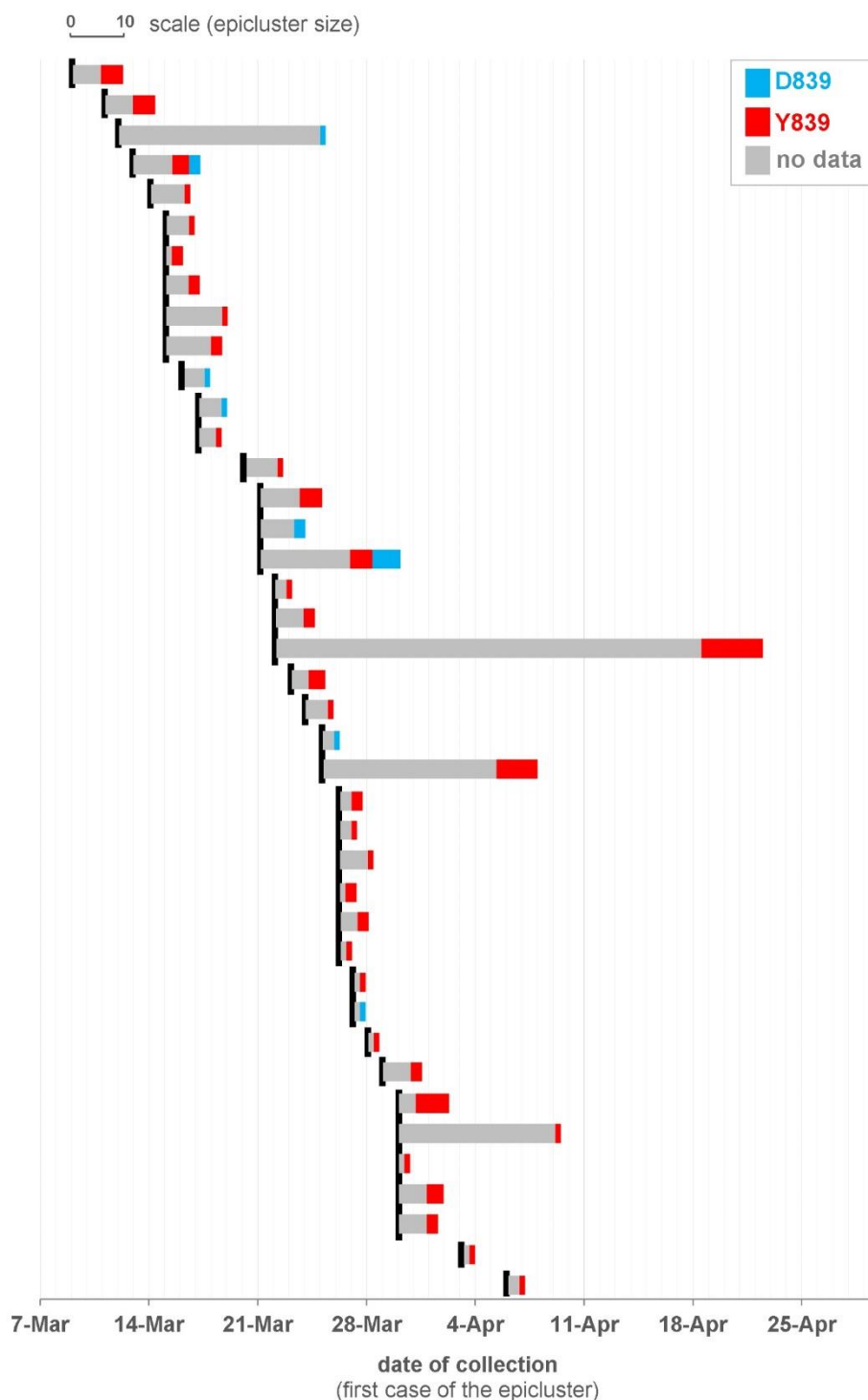


Figure S2. SARS-CoV-2 D839/Y839 status of epiclusters of potential epidemiologically linked confirmed cases (“epiclusters”) monitored by the Public Health Unit of Primary Care Cluster of Baixo Vouga, as of April 30th, 2020. The graph shows the temporal distribution (by date of sample collection of the first case of the epicluster; black lines) of 41 potential “epiclusters” (covering a total of 420 confirmed cases) for which SARS-CoV-2 genome data is already available. 33 epiclusters (323 confirmed cases) (77%) are exclusively associated with the Spike Y839 variant. The two potential epiclusters where both D839 and Y839 variants were ambiguously detected are under close contact tracing investigation to disclose this incongruence.

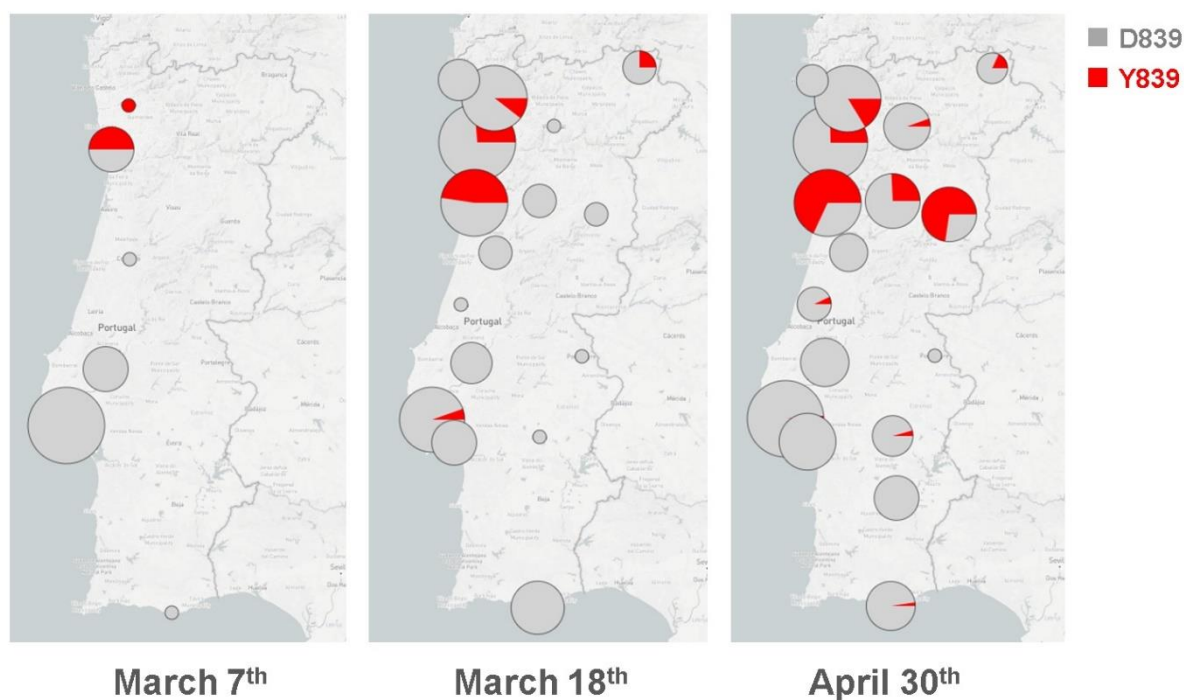


Figure S3. Geographical distribution of the relative frequencies of the D839 (gray) and Y839 (red) variants by District in three timeframes: March 7th (when the first Y839 genomes were detected), **March 18th** (when the emergency state was declared in Portugal and national lockdown was implemented) **and April 30th, 2020**. Autonomous regions (Azores and Madeira) are not shown in these maps as no Y839 case where detected there (see Figure S1). The phylogeny and geotemporal distribution can be visualized interactively at <https://microreact.org/project/nDGsJKFv7gQTj1q8CQwwKR/0489f840> (geographic resolution by Region) and <https://microreact.org/project/2kh3TRVYB9gWGRpNSJWDW5/b6c659e0> (geographic resolution by District) using Microreact (<https://microreact.org/>).

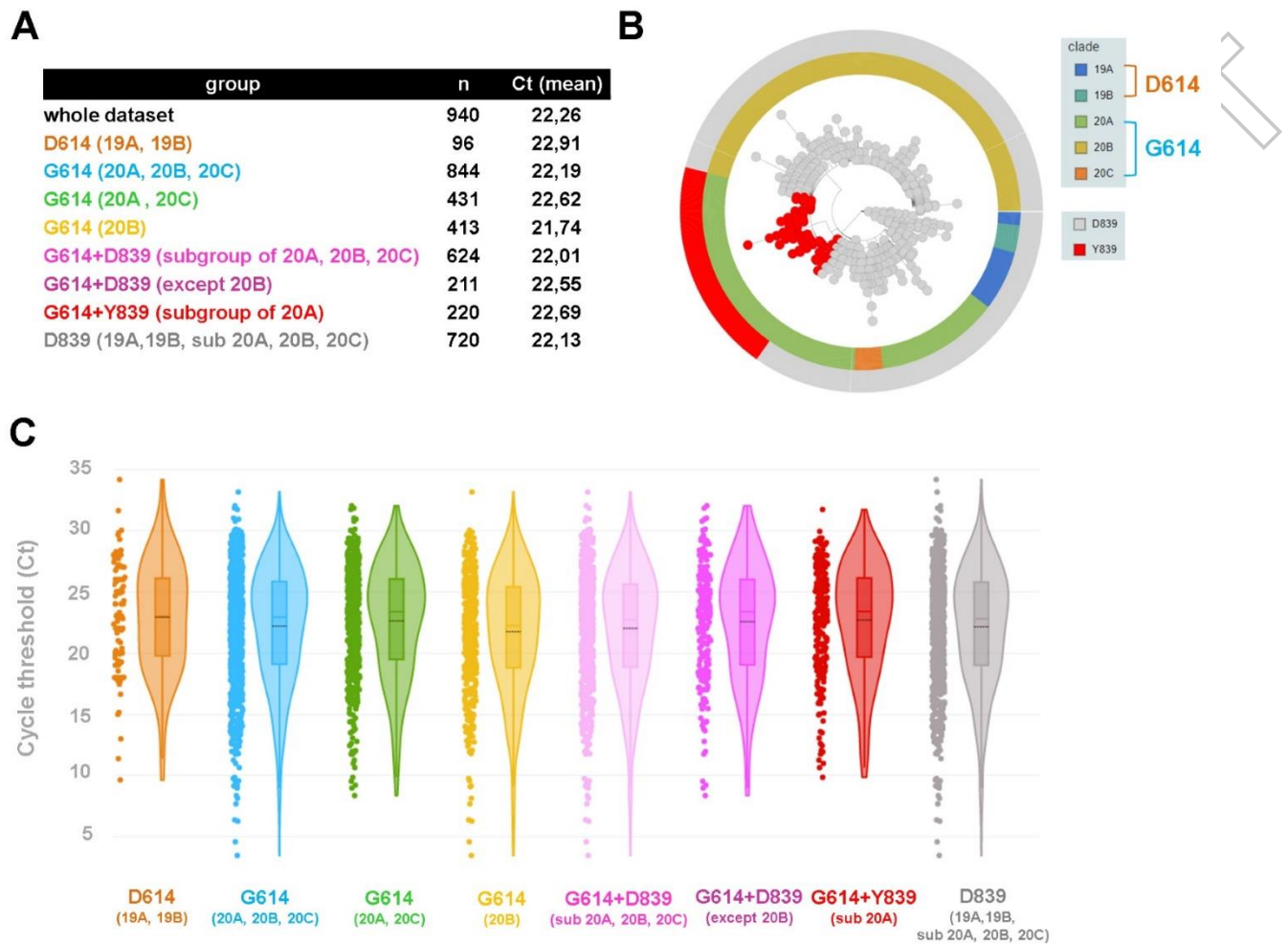


Figure S4. Comparison of Cycle threshold (Ct) values obtained in diagnostic PCR between different samples groups. Groups were established according to phylogenetic clustering and Nextstrain Clade classification of the genomes studied, D614/G614 status, phylogenetic group within G614 (i.e., 20B or non-20B) and Spike D839/Y839 status. **A.** Mean Ct values observed per group. **B.** Global phylogeny of the 1516 genomes studied (<https://microreact.org/project/nDGsJKFv7gQTj1q8CQwwKR/f46f1fa4>) highlighting the Nextstrain clades, D614/G614 and D839/Y839 status. **C.** Violin, scatter and box plots showing the dispersion of Ct values per group. Mean Ct values are indicated by black dash lines.

Table S1. List of SARS-CoV-2 genome sequences from Portugal used in this study.

Sample designation	Location	Spike Y839 variant	Collection date	GISAID Accession ID
Portugal/CV62/2020	Europe / Portugal		2020-03-01	EPI_ISL_413647
Portugal/CV63/2020	Europe / Portugal		2020-03-01	EPI_ISL_413648
Portugal/PT0003/2020	Europe / Portugal		2020-03-03	EPI_ISL_417987
Portugal/PT0025/2020	Europe / Portugal		2020-03-03	EPI_ISL_418010
Portugal/PT0005/2020	Europe / Portugal		2020-03-04	EPI_ISL_417989
Portugal/PT0026/2020	Europe / Portugal		2020-03-04	EPI_ISL_418011
Portugal/PT0004/2020	Europe / Portugal		2020-03-05	EPI_ISL_417988
Portugal/PT0006a/2020	Europe / Portugal		2020-03-06	EPI_ISL_417990
Portugal/PT0027/2020	Europe / Portugal		2020-03-06	EPI_ISL_418012
Portugal/PT0007/2020	Europe / Portugal		2020-03-07	EPI_ISL_417992
Portugal/PT0012/2020	Europe / Portugal	yes	2020-03-07	EPI_ISL_417997
Portugal/PT0014/2020	Europe / Portugal	yes	2020-03-07	EPI_ISL_417999
Portugal/PT0028/2020	Europe / Portugal		2020-03-07	EPI_ISL_418013
Portugal/PT0008/2020	Europe / Portugal		2020-03-08	EPI_ISL_417993
Portugal/PT0009/2020	Europe / Portugal		2020-03-08	EPI_ISL_417994
Portugal/PT0010/2020	Europe / Portugal		2020-03-08	EPI_ISL_417995
Portugal/PT0011/2020	Europe / Portugal		2020-03-08	EPI_ISL_417996
Portugal/PT0013/2020	Europe / Portugal	yes	2020-03-08	EPI_ISL_417998
Portugal/PT1516/2020	Europe / Portugal	yes	2020-03-08	EPI_ISL_511475
Portugal/PT0029/2020	Europe / Portugal		2020-03-09	EPI_ISL_418014
Portugal/PT0030/2020	Europe / Portugal		2020-03-09	EPI_ISL_418015
Portugal/PT0285/2020	Europe / Portugal		2020-03-09	EPI_ISL_454000
Portugal/PT0286a/2020	Europe / Portugal		2020-03-09	EPI_ISL_454001
Portugal/PT0287/2020	Europe / Portugal		2020-03-09	EPI_ISL_454003
Portugal/PT1517/2020	Europe / Portugal		2020-03-09	EPI_ISL_511476
Portugal/PT0015/2020	Europe / Portugal		2020-03-10	EPI_ISL_418000
Portugal/PT0016/2020	Europe / Portugal		2020-03-10	EPI_ISL_418001
Portugal/PT0018/2020	Europe / Portugal	yes	2020-03-10	EPI_ISL_418003
Portugal/PT0031/2020	Europe / Portugal		2020-03-10	EPI_ISL_418016
Portugal/PT0288/2020	Europe / Portugal	yes	2020-03-10	EPI_ISL_454004
Portugal/PT0289/2020	Europe / Portugal		2020-03-10	EPI_ISL_454005
Portugal/PT0017/2020	Europe / Portugal		2020-03-11	EPI_ISL_418002
Portugal/PT0291/2020	Europe / Portugal		2020-03-11	EPI_ISL_454007
Portugal/PT0498/2020	Europe / Portugal		2020-03-11	EPI_ISL_454222
Portugal/PT0019/2020	Europe / Portugal		2020-03-12	EPI_ISL_418004
Portugal/PT0020/2020	Europe / Portugal		2020-03-12	EPI_ISL_418005
Portugal/PT0290/2020	Europe / Portugal	yes	2020-03-12	EPI_ISL_454006
Portugal/PT0292/2020	Europe / Portugal		2020-03-12	EPI_ISL_454008
Portugal/PT0293/2020	Europe / Portugal	yes	2020-03-12	EPI_ISL_454009
Portugal/PT0294/2020	Europe / Portugal	yes	2020-03-12	EPI_ISL_454010
Portugal/PT0021/2020	Europe / Portugal		2020-03-13	EPI_ISL_418006
Portugal/PT0022/2020	Europe / Portugal		2020-03-13	EPI_ISL_418007
Portugal/PT0023/2020	Europe / Portugal		2020-03-13	EPI_ISL_418008
Portugal/PT0295/2020	Europe / Portugal		2020-03-13	EPI_ISL_454011
Portugal/PT0296/2020	Europe / Portugal		2020-03-13	EPI_ISL_454012
Portugal/PT0297/2020	Europe / Portugal		2020-03-13	EPI_ISL_454013
Portugal/PT0298/2020	Europe / Portugal	yes	2020-03-13	EPI_ISL_454014
Portugal/PT0299/2020	Europe / Portugal		2020-03-13	EPI_ISL_454015
Portugal/PT0300/2020	Europe / Portugal		2020-03-13	EPI_ISL_454016
Portugal/PT0499/2020	Europe / Portugal		2020-03-13	EPI_ISL_454223
Portugal/PT0606/2020	Europe / Portugal		2020-03-13	EPI_ISL_454329
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Portugal/PT1509a/2020	Europe / Portugal		2020-03-13	EPI_ISL_511468
Portugal/PT0301/2020	Europe / Portugal	yes	2020-03-14	EPI_ISL_454017
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Portugal/PT0305/2020	Europe / Portugal		2020-03-14	EPI_ISL_454021
Portugal/PT0306/2020	Europe / Portugal		2020-03-14	EPI_ISL_454022
Portugal/PT0307/2020	Europe / Portugal		2020-03-14	EPI_ISL_454023
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Portugal/PT0310/2020	Europe / Portugal	yes	2020-03-14	EPI_ISL_454026
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Portugal/PT0315/2020	Europe / Portugal		2020-03-14	EPI_ISL_454031
Portugal/PT0316/2020	Europe / Portugal		2020-03-14	EPI_ISL_454032
Portugal/PT0342/2020	Europe / Portugal		2020-03-14	EPI_ISL_454058
Portugal/PT0500/2020	Europe / Portugal		2020-03-14	EPI_ISL_454224
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Portugal/PT0608/2020	Europe / Portugal		2020-03-14	EPI_ISL_454331
Portugal/PT0609/2020	Europe / Portugal		2020-03-14	EPI_ISL_454332
Portugal/PT0610/2020	Europe / Portugal		2020-03-14	EPI_ISL_454333
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Portugal/PT0613/2020	Europe / Portugal	yes	2020-03-15	EPI_ISL_454336
Portugal/PT0781/2020	Europe / Portugal	yes	2020-03-15	EPI_ISL_511040
Portugal/PT0782/2020	Europe / Portugal		2020-03-15	EPI_ISL_511041
Portugal/PT1475/2020	Europe / Portugal		2020-03-15	EPI_ISL_511434
Portugal/PT1493/2020	Europe / Portugal	yes	2020-03-15	EPI_ISL_511452
Portugal/PT1501/2020	Europe / Portugal	yes	2020-03-15	EPI_ISL_511460
Portugal/PT1514/2020	Europe / Portugal		2020-03-15	EPI_ISL_511473
Portugal/PT1515/2020	Europe / Portugal		2020-03-15	EPI_ISL_511474
Portugal/PT0036a/2020	Europe / Portugal		2020-03-15	EPI_ISL_511189
Portugal/PT0032/2020	Europe / Portugal		2020-03-16	EPI_ISL_418017
Portugal/PT0033/2020	Europe / Portugal		2020-03-16	EPI_ISL_418018
Portugal/PT0035/2020	Europe / Portugal		2020-03-16	EPI_ISL_418020
Portugal/PT0038/2020	Europe / Portugal		2020-03-16	EPI_ISL_418023
Portugal/PT0302/2020	Europe / Portugal	yes	2020-03-16	EPI_ISL_454018
Portugal/PT0314/2020	Europe / Portugal	yes	2020-03-16	EPI_ISL_454030
Portugal/PT0318/2020	Europe / Portugal	yes	2020-03-16	EPI_ISL_454034
Portugal/PT0322/2020	Europe / Portugal	yes	2020-03-16	EPI_ISL_454038
Portugal/PT0325/2020	Europe / Portugal		2020-03-16	EPI_ISL_454041
Portugal/PT0326/2020	Europe / Portugal		2020-03-16	EPI_ISL_454042
Portugal/PT0327/2020	Europe / Portugal	yes	2020-03-16	EPI_ISL_454043
Portugal/PT0328/2020	Europe / Portugal		2020-03-16	EPI_ISL_454044
Portugal/PT0331/2020	Europe / Portugal		2020-03-16	EPI_ISL_454047
Portugal/PT0614/2020	Europe / Portugal		2020-03-16	EPI_ISL_454337
Portugal/PT0778/2020	Europe / Portugal		2020-03-16	EPI_ISL_511037
Portugal/PT0783/2020	Europe / Portugal		2020-03-16	EPI_ISL_511042
Portugal/PT0784/2020	Europe / Portugal		2020-03-16	EPI_ISL_511043
Portugal/PT1095/2020	Europe / Portugal		2020-03-16	EPI_ISL_511642

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Portugal/PT1356/2020	Europe / Portugal	yes	2020-03-25	EPI_ISL_511315
Portugal/PT1357/2020	Europe / Portugal		2020-03-25	EPI_ISL_511316
Portugal/PT1358/2020	Europe / Portugal		2020-03-25	EPI_ISL_511317
Portugal/PT1371/2020	Europe / Portugal		2020-03-25	EPI_ISL_511330
Portugal/PT1372/2020	Europe / Portugal		2020-03-25	EPI_ISL_511331
Portugal/PT1373/2020	Europe / Portugal		2020-03-25	EPI_ISL_511332
Portugal/PT1374/2020	Europe / Portugal		2020-03-25	EPI_ISL_511333
Portugal/PT1375/2020	Europe / Portugal		2020-03-25	EPI_ISL_511334
Portugal/PT1407/2020	Europe / Portugal		2020-03-25	EPI_ISL_511366
Portugal/PT1410/2020	Europe / Portugal		2020-03-25	EPI_ISL_511369
Portugal/PT1412/2020	Europe / Portugal	yes	2020-03-25	EPI_ISL_511371
Portugal/PT1419/2020	Europe / Portugal		2020-03-25	EPI_ISL_511378
Portugal/PT1427/2020	Europe / Portugal		2020-03-25	EPI_ISL_511386
Portugal/PT1442/2020	Europe / Portugal		2020-03-25	EPI_ISL_511401
Portugal/PT1444/2020	Europe / Portugal		2020-03-25	EPI_ISL_511403
Portugal/PT0121/2020	Europe / Portugal		2020-03-26	EPI_ISL_453837
Portugal/PT0122/2020	Europe / Portugal		2020-03-26	EPI_ISL_453838
Portugal/PT0126/2020	Europe / Portugal		2020-03-26	EPI_ISL_453842
Portugal/PT0133/2020	Europe / Portugal		2020-03-26	EPI_ISL_453849
Portugal/PT0134/2020	Europe / Portugal		2020-03-26	EPI_ISL_453850
Portugal/PT0135/2020	Europe / Portugal		2020-03-26	EPI_ISL_453851
Portugal/PT0136/2020	Europe / Portugal		2020-03-26	EPI_ISL_453852
Portugal/PT0137/2020	Europe / Portugal		2020-03-26	EPI_ISL_453853
Portugal/PT0216/2020	Europe / Portugal		2020-03-26	EPI_ISL_453930
Portugal/PT0444/2020	Europe / Portugal		2020-03-26	EPI_ISL_454168
Portugal/PT0445/2020	Europe / Portugal		2020-03-26	EPI_ISL_454169
Portugal/PT0564/2020	Europe / Portugal		2020-03-26	EPI_ISL_454287
Portugal/PT0565/2020	Europe / Portugal		2020-03-26	EPI_ISL_454288
Portugal/PT0571/2020	Europe / Portugal		2020-03-26	EPI_ISL_454294
Portugal/PT0572/2020	Europe / Portugal		2020-03-26	EPI_ISL_454295
Portugal/PT0582/2020	Europe / Portugal		2020-03-26	EPI_ISL_454305
Portugal/PT0603/2020	Europe / Portugal		2020-03-26	EPI_ISL_454326
Portugal/PT0622/2020	Europe / Portugal		2020-03-26	EPI_ISL_454345
Portugal/PT0623/2020	Europe / Portugal	yes	2020-03-26	EPI_ISL_454346
Portugal/PT0624/2020	Europe / Portugal		2020-03-26	EPI_ISL_454347
Portugal/PT0654/2020	Europe / Portugal	yes	2020-03-26	EPI_ISL_510915
Portugal/PT0666/2020	Europe / Portugal		2020-03-26	EPI_ISL_510925
Portugal/PT0720/2020	Europe / Portugal		2020-03-26	EPI_ISL_510979
Portugal/PT0721/2020	Europe / Portugal		2020-03-26	EPI_ISL_510980
Portugal/PT0768/2020	Europe / Portugal	yes	2020-03-26	EPI_ISL_511027
Portugal/PT0897/2020	Europe / Portugal		2020-03-26	EPI_ISL_511154
Portugal/PT0932/2020	Europe / Portugal		2020-03-26	EPI_ISL_511484
Portugal/PT0960/2020	Europe / Portugal		2020-03-26	EPI_ISL_511509
Portugal/PT0963/2020	Europe / Portugal		2020-03-26	EPI_ISL_511512
Portugal/PT1005/2020	Europe / Portugal	yes	2020-03-26	EPI_ISL_511552
Portugal/PT1012/2020	Europe / Portugal		2020-03-26	EPI_ISL_511559
Portugal/PT1013/2020	Europe / Portugal	yes	2020-03-26	EPI_ISL_511560
Portugal/PT1014/2020	Europe / Portugal	yes	2020-03-26	EPI_ISL_511561
Portugal/PT1015/2020	Europe / Portugal	yes	2020-03-26	EPI_ISL_511562
Portugal/PT1016/2020	Europe / Portugal		2020-03-26	EPI_ISL_511563
Portugal/PT1018/2020	Europe / Portugal		2020-03-26	EPI_ISL_511565
Portugal/PT1019/2020	Europe / Portugal		2020-03-26	EPI_ISL_511566
Portugal/PT1020/2020	Europe / Portugal	yes	2020-03-26	EPI_ISL_511567
Portugal/PT1021/2020	Europe / Portugal		2020-03-26	EPI_ISL_511568
Portugal/PT1025/2020	Europe / Portugal		2020-03-26	EPI_ISL_511572
Portugal/PT1029/2020	Europe / Portugal		2020-03-26	EPI_ISL_511576
Portugal/PT1034/2020	Europe / Portugal		2020-03-26	EPI_ISL_511581
Portugal/PT1035/2020	Europe / Portugal		2020-03-26	EPI_ISL_511582
Portugal/PT1036/2020	Europe / Portugal		2020-03-26	EPI_ISL_511583
Portugal/PT1037/2020	Europe / Portugal		2020-03-26	EPI_ISL_511584
Portugal/PT1125/2020	Europe / Portugal		2020-03-26	EPI_ISL_511672
Portugal/PT1126/2020	Europe / Portugal		2020-03-26	EPI_ISL_511673
Portugal/PT1187/2020	Europe / Portugal		2020-03-26	EPI_ISL_511732
Portugal/PT1188/2020	Europe / Portugal		2020-03-26	EPI_ISL_511733
Portugal/PT1208/2020	Europe / Portugal		2020-03-26	EPI_ISL_511751

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Portugal/PT0203/2020	Europe / Portugal		2020-03-29	EPI_ISL_453917
Portugal/PT0482/2020	Europe / Portugal	yes	2020-03-29	EPI_ISL_454206
Portugal/PT0574/2020	Europe / Portugal		2020-03-29	EPI_ISL_454297
Portugal/PT0600/2020	Europe / Portugal		2020-03-29	EPI_ISL_454323
Portugal/PT0626/2020	Europe / Portugal	yes	2020-03-29	EPI_ISL_454349
Portugal/PT0640/2020	Europe / Portugal		2020-03-29	EPI_ISL_510901
Portugal/PT0641/2020	Europe / Portugal	yes	2020-03-29	EPI_ISL_510902
Portugal/PT0642/2020	Europe / Portugal		2020-03-29	EPI_ISL_510903
Portugal/PT0643/2020	Europe / Portugal		2020-03-29	EPI_ISL_510904
Portugal/PT0644/2020	Europe / Portugal		2020-03-29	EPI_ISL_510905
Portugal/PT0645/2020	Europe / Portugal	yes	2020-03-29	EPI_ISL_510906
Portugal/PT0660/2020	Europe / Portugal		2020-03-29	EPI_ISL_510919
Portugal/PT0661/2020	Europe / Portugal	yes	2020-03-29	EPI_ISL_510920
Portugal/PT0919/2020	Europe / Portugal		2020-03-29	EPI_ISL_511176
Portugal/PT0920/2020	Europe / Portugal		2020-03-29	EPI_ISL_511177
Portugal/PT0967/2020	Europe / Portugal		2020-03-29	EPI_ISL_511516
Portugal/PT0968/2020	Europe / Portugal		2020-03-29	EPI_ISL_511517
Portugal/PT0972/2020	Europe / Portugal		2020-03-29	EPI_ISL_511521
Portugal/PT1056/2020	Europe / Portugal		2020-03-29	EPI_ISL_511603
Portugal/PT1057/2020	Europe / Portugal		2020-03-29	EPI_ISL_511604
Portugal/PT1058/2020	Europe / Portugal		2020-03-29	EPI_ISL_511605
Portugal/PT1060/2020	Europe / Portugal	yes	2020-03-29	EPI_ISL_511607
Portugal/PT1061/2020	Europe / Portugal	yes	2020-03-29	EPI_ISL_511608
Portugal/PT1101/2020	Europe / Portugal		2020-03-29	EPI_ISL_511648
Portugal/PT0530a/2020	Europe / Portugal	yes	2020-03-29	EPI_ISL_511480
Portugal/PT1191/2020	Europe / Portugal		2020-03-29	EPI_ISL_582520
Portugal/PT1223/2020	Europe / Portugal		2020-03-29	EPI_ISL_511766
Portugal/PT1224/2020	Europe / Portugal		2020-03-29	EPI_ISL_511767
Portugal/PT1225/2020	Europe / Portugal		2020-03-29	EPI_ISL_511768
Portugal/PT1226/2020	Europe / Portugal		2020-03-29	EPI_ISL_511769
Portugal/PT1228/2020	Europe / Portugal		2020-03-29	EPI_ISL_511771
Portugal/PT1229/2020	Europe / Portugal		2020-03-29	EPI_ISL_511190
Portugal/PT1401/2020	Europe / Portugal		2020-03-29	EPI_ISL_511360
Portugal/PT1445/2020	Europe / Portugal		2020-03-29	EPI_ISL_511404
Portugal/PT1460/2020	Europe / Portugal		2020-03-29	EPI_ISL_511419
Portugal/PT1507/2020	Europe / Portugal		2020-03-29	EPI_ISL_511466
Portugal/PT0206/2020	Europe / Portugal		2020-03-30	EPI_ISL_453920
Portugal/PT0207/2020	Europe / Portugal		2020-03-30	EPI_ISL_453921
Portugal/PT0208/2020	Europe / Portugal		2020-03-30	EPI_ISL_453922
Portugal/PT0209/2020	Europe / Portugal		2020-03-30	EPI_ISL_453923
Portugal/PT0210/2020	Europe / Portugal		2020-03-30	EPI_ISL_453924
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Portugal/PT0213/2020	Europe / Portugal		2020-03-30	EPI_ISL_453927
Portugal/PT0214/2020	Europe / Portugal		2020-03-30	EPI_ISL_453928
Portugal/PT0215/2020	Europe / Portugal		2020-03-30	EPI_ISL_453929
Portugal/PT0217/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_453931
Portugal/PT0218/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_453932
Portugal/PT0219/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_453933
Portugal/PT0220/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_453934
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Portugal/PT0222/2020	Europe / Portugal		2020-03-30	EPI_ISL_453936
Portugal/PT0223/2020	Europe / Portugal		2020-03-30	EPI_ISL_453937
Portugal/PT0483/2020	Europe / Portugal		2020-03-30	EPI_ISL_454207
Portugal/PT0485/2020	Europe / Portugal		2020-03-30	EPI_ISL_454209
Portugal/PT0486/2020	Europe / Portugal		2020-03-30	EPI_ISL_454210
Portugal/PT0592/2020	Europe / Portugal		2020-03-30	EPI_ISL_454315
Portugal/PT0646/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_510907
Portugal/PT0647/2020	Europe / Portugal		2020-03-30	EPI_ISL_510908
Portugal/PT0685/2020	Europe / Portugal		2020-03-30	EPI_ISL_510944
Portugal/PT0686/2020	Europe / Portugal		2020-03-30	EPI_ISL_510945
Portugal/PT0977/2020	Europe / Portugal		2020-03-30	EPI_ISL_511526

Portugal/PT0978/2020	Europe / Portugal		2020-03-30	EPI_ISL_511527
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Portugal/PT1063/2020	Europe / Portugal		2020-03-30	EPI_ISL_511610
Portugal/PT1110/2020	Europe / Portugal		2020-03-30	EPI_ISL_511657
Portugal/PT1117/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_511664
Portugal/PT1118/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_511665
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Portugal/PT1120/2020	Europe / Portugal		2020-03-30	EPI_ISL_511667
Portugal/PT1123/2020	Europe / Portugal		2020-03-30	EPI_ISL_511670
Portugal/PT1192/2020	Europe / Portugal		2020-03-30	EPI_ISL_511736
Portugal/PT1205/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_511748
Portugal/PT1206/2020	Europe / Portugal		2020-03-30	EPI_ISL_511749
Portugal/PT1207/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_511750
Portugal/PT1230/2020	Europe / Portugal		2020-03-30	EPI_ISL_511191
Portugal/PT1416/2020	Europe / Portugal		2020-03-30	EPI_ISL_511375
Portugal/PT1428/2020	Europe / Portugal		2020-03-30	EPI_ISL_511387
Portugal/PT1436/2020	Europe / Portugal		2020-03-30	EPI_ISL_511395
Portugal/PT1448/2020	Europe / Portugal		2020-03-30	EPI_ISL_511407
Portugal/PT1455/2020	Europe / Portugal		2020-03-30	EPI_ISL_511414
Portugal/PT1456/2020	Europe / Portugal		2020-03-30	EPI_ISL_511415
Portugal/PT1463/2020	Europe / Portugal		2020-03-30	EPI_ISL_511422
Portugal/PT1465/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_511424
Portugal/PT1477/2020	Europe / Portugal		2020-03-30	EPI_ISL_511436
Portugal/PT1480/2020	Europe / Portugal		2020-03-30	EPI_ISL_511439
Portugal/PT1483/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_511442
Portugal/PT1494/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_511453
Portugal/PT1498/2020	Europe / Portugal	yes	2020-03-30	EPI_ISL_511457
Portugal/PT1502/2020	Europe / Portugal		2020-03-30	EPI_ISL_511461
Portugal/PT0224/2020	Europe / Portugal		2020-03-31	EPI_ISL_453938
Portugal/PT0225/2020	Europe / Portugal		2020-03-31	EPI_ISL_453939
Portugal/PT0226/2020	Europe / Portugal		2020-03-31	EPI_ISL_453940
Portugal/PT0227/2020	Europe / Portugal		2020-03-31	EPI_ISL_453941
Portugal/PT0228/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453942
Portugal/PT0229/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453943
Portugal/PT0230/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453944
Portugal/PT0231/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453945
Portugal/PT0232/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453946
Portugal/PT0233/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453947
Portugal/PT0234/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453948
Portugal/PT0235/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453949
Portugal/PT0236/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453950
Portugal/PT0237/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453951
Portugal/PT0238/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453952
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Portugal/PT0242/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_453956
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Portugal/PT0244/2020	Europe / Portugal		2020-03-31	EPI_ISL_453958
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Portugal/PT0650/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_510911
Portugal/PT0651/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_510912
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Portugal/PT0687/2020	Europe / Portugal		2020-03-31	EPI_ISL_510946
Portugal/PT0921/2020	Europe / Portugal	yes	2020-03-31	EPI_ISL_511178
Portugal/PT0980/2020	Europe / Portugal		2020-03-31	EPI_ISL_511529
Portugal/PT1109/2020	Europe / Portugal		2020-03-31	EPI_ISL_511656

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Portugal/PT1162/2020	Europe / Portugal		2020-04-02	EPI_ISL_511709
Portugal/PT1163/2020	Europe / Portugal		2020-04-02	EPI_ISL_511710
Portugal/PT0253/2020	Europe / Portugal		2020-04-03	EPI_ISL_453967
Portugal/PT0254/2020	Europe / Portugal	yes	2020-04-03	EPI_ISL_453968
Portugal/PT0255/2020	Europe / Portugal	yes	2020-04-03	EPI_ISL_453969
Portugal/PT0256/2020	Europe / Portugal	yes	2020-04-03	EPI_ISL_453970
Portugal/PT0257/2020	Europe / Portugal		2020-04-03	EPI_ISL_453971
Portugal/PT0258/2020	Europe / Portugal		2020-04-03	EPI_ISL_453972
Portugal/PT0259/2020	Europe / Portugal		2020-04-03	EPI_ISL_453973
Portugal/PT0260/2020	Europe / Portugal		2020-04-03	EPI_ISL_453974
Portugal/PT0261/2020	Europe / Portugal		2020-04-03	EPI_ISL_453975
Portugal/PT0262/2020	Europe / Portugal		2020-04-03	EPI_ISL_453976
Portugal/PT0451/2020	Europe / Portugal		2020-04-03	EPI_ISL_454175
Portugal/PT0547/2020	Europe / Portugal	yes	2020-04-03	EPI_ISL_454271
Portugal/PT0549/2020	Europe / Portugal		2020-04-03	EPI_ISL_455627
Portugal/PT0594/2020	Europe / Portugal		2020-04-03	EPI_ISL_454317
Portugal/PT0774/2020	Europe / Portugal		2020-04-03	EPI_ISL_511033
Portugal/PT0928/2020	Europe / Portugal		2020-04-03	EPI_ISL_511185
Portugal/PT0936/2020	Europe / Portugal		2020-04-03	EPI_ISL_511487
Portugal/PT0937/2020	Europe / Portugal		2020-04-03	EPI_ISL_511488
Portugal/PT0938/2020	Europe / Portugal		2020-04-03	EPI_ISL_511489
Portugal/PT0263/2020	Europe / Portugal		2020-04-04	EPI_ISL_453977
Portugal/PT0264/2020	Europe / Portugal		2020-04-04	EPI_ISL_453978
Portugal/PT0265/2020	Europe / Portugal	yes	2020-04-04	EPI_ISL_453979
Portugal/PT0266/2020	Europe / Portugal	yes	2020-04-04	EPI_ISL_453980
Portugal/PT0267/2020	Europe / Portugal		2020-04-04	EPI_ISL_453981
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Portugal/PT0663/2020	Europe / Portugal	yes	2020-04-04	EPI_ISL_510922
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Portugal/PT0270/2020	Europe / Portugal		2020-04-05	EPI_ISL_453984
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Portugal/PT0273/2020	Europe / Portugal		2020-04-05	EPI_ISL_453987
Portugal/PT0274/2020	Europe / Portugal		2020-04-05	EPI_ISL_453988
Portugal/PT0275/2020	Europe / Portugal		2020-04-05	EPI_ISL_453989
Portugal/PT0276/2020	Europe / Portugal		2020-04-05	EPI_ISL_453990
Portugal/PT0277/2020	Europe / Portugal		2020-04-05	EPI_ISL_453991
Portugal/PT0278/2020	Europe / Portugal		2020-04-05	EPI_ISL_453992
Portugal/PT0279/2020	Europe / Portugal		2020-04-05	EPI_ISL_453993
Portugal/PT0280/2020	Europe / Portugal		2020-04-05	EPI_ISL_453994
Portugal/PT0281/2020	Europe / Portugal		2020-04-05	EPI_ISL_453995
Portugal/PT0282/2020	Europe / Portugal		2020-04-05	EPI_ISL_453996
Portugal/PT1166/2020	Europe / Portugal		2020-04-05	EPI_ISL_511712
Portugal/PT1167/2020	Europe / Portugal		2020-04-05	EPI_ISL_511713
Portugal/PT1168/2020	Europe / Portugal		2020-04-05	EPI_ISL_511714
Portugal/PT1169/2020	Europe / Portugal		2020-04-05	EPI_ISL_511715
Portugal/PT0526/2020	Europe / Portugal		2020-04-06	EPI_ISL_454250
Portugal/PT0527/2020	Europe / Portugal	yes	2020-04-06	EPI_ISL_454251
Portugal/PT0528/2020	Europe / Portugal	yes	2020-04-06	EPI_ISL_454252
Portugal/PT0529/2020	Europe / Portugal		2020-04-06	EPI_ISL_454253
Portugal/PT0601/2020	Europe / Portugal		2020-04-06	EPI_ISL_454324
Portugal/PT0628/2020	Europe / Portugal		2020-04-06	EPI_ISL_454351
Portugal/PT0629/2020	Europe / Portugal		2020-04-06	EPI_ISL_454352
Portugal/PT1164/2020	Europe / Portugal		2020-04-06	EPI_ISL_582518
Portugal/PT1165/2020	Europe / Portugal		2020-04-06	EPI_ISL_511711
Portugal/PT1402/2020	Europe / Portugal		2020-04-06	EPI_ISL_511361
Portugal/PT0531/2020	Europe / Portugal		2020-04-07	EPI_ISL_454255
Portugal/PT0532/2020	Europe / Portugal		2020-04-07	EPI_ISL_454256
Portugal/PT0533/2020	Europe / Portugal		2020-04-07	EPI_ISL_454257

Portugal/PT0534/2020	Europe / Portugal		2020-04-07	EPI_ISL_454258
Portugal/PT0535/2020	Europe / Portugal		2020-04-07	EPI_ISL_454259
Portugal/PT0536/2020	Europe / Portugal	yes	2020-04-07	EPI_ISL_454260
Portugal/PT0537/2020	Europe / Portugal	yes	2020-04-07	EPI_ISL_454261
Portugal/PT0538/2020	Europe / Portugal		2020-04-07	EPI_ISL_454262
Portugal/PT0539/2020	Europe / Portugal	yes	2020-04-07	EPI_ISL_454263
Portugal/PT0551/2020	Europe / Portugal	yes	2020-04-07	EPI_ISL_454274
Portugal/PT0552/2020	Europe / Portugal	yes	2020-04-07	EPI_ISL_454275
Portugal/PT0553/2020	Europe / Portugal	yes	2020-04-07	EPI_ISL_454276
Portugal/PT0554/2020	Europe / Portugal	yes	2020-04-07	EPI_ISL_454277
Portugal/PT0540/2020	Europe / Portugal		2020-04-08	EPI_ISL_454264
Portugal/PT0541/2020	Europe / Portugal		2020-04-08	EPI_ISL_454265
Portugal/PT0542/2020	Europe / Portugal		2020-04-08	EPI_ISL_454266
Portugal/PT0543/2020	Europe / Portugal		2020-04-08	EPI_ISL_454267
Portugal/PT0555/2020	Europe / Portugal	yes	2020-04-08	EPI_ISL_454278
Portugal/PT0770a/2020	Europe / Portugal		2020-04-08	EPI_ISL_511029
Portugal/PT0939/2020	Europe / Portugal		2020-04-08	EPI_ISL_582514
Portugal/PT0940/2020	Europe / Portugal		2020-04-08	EPI_ISL_511490
Portugal/PT0941/2020	Europe / Portugal		2020-04-08	EPI_ISL_511491
Portugal/PT1144/2020	Europe / Portugal		2020-04-08	EPI_ISL_511691
Portugal/PT1145/2020	Europe / Portugal		2020-04-08	EPI_ISL_511692
Portugal/PT1146/2020	Europe / Portugal		2020-04-08	EPI_ISL_511693
Portugal/PT1147/2020	Europe / Portugal		2020-04-08	EPI_ISL_511694
Portugal/PT1148/2020	Europe / Portugal		2020-04-08	EPI_ISL_511695
Portugal/PT1149/2020	Europe / Portugal		2020-04-08	EPI_ISL_511696
Portugal/PT0544/2020	Europe / Portugal	yes	2020-04-09	EPI_ISL_454268
Portugal/PT0602/2020	Europe / Portugal		2020-04-09	EPI_ISL_454325
Portugal/PT0942/2020	Europe / Portugal		2020-04-09	EPI_ISL_511492
Portugal/PT0943/2020	Europe / Portugal		2020-04-09	EPI_ISL_511493
Portugal/PT0944/2020	Europe / Portugal		2020-04-09	EPI_ISL_511494
Portugal/PT1030/2020	Europe / Portugal		2020-04-09	EPI_ISL_511577
Portugal/PT0568a/2020	Europe / Portugal		2020-04-09	EPI_ISL_511481
Portugal/PT0545/2020	Europe / Portugal		2020-04-10	EPI_ISL_454269
Portugal/PT0566/2020	Europe / Portugal	yes	2020-04-10	EPI_ISL_454289
Portugal/PT0945/2020	Europe / Portugal		2020-04-10	EPI_ISL_511495
Portugal/PT1130/2020	Europe / Portugal	yes	2020-04-10	EPI_ISL_511677
Portugal/PT1131/2020	Europe / Portugal		2020-04-10	EPI_ISL_511678
Portugal/PT0573/2020	Europe / Portugal		2020-04-11	EPI_ISL_454296
Portugal/PT0575/2020	Europe / Portugal		2020-04-11	EPI_ISL_454298
Portugal/PT0946/2020	Europe / Portugal		2020-04-11	EPI_ISL_511496
Portugal/PT0947/2020	Europe / Portugal		2020-04-11	EPI_ISL_511497
Portugal/PT1097/2020	Europe / Portugal		2020-04-11	EPI_ISL_511644
Portugal/PT0948/2020	Europe / Portugal		2020-04-12	EPI_ISL_511498
Portugal/PT0949/2020	Europe / Portugal		2020-04-12	EPI_ISL_511499
Portugal/PT0950/2020	Europe / Portugal		2020-04-12	EPI_ISL_511500
Portugal/PT0576/2020	Europe / Portugal		2020-04-13	EPI_ISL_454299
Portugal/PT0401/2020	Europe / Portugal	yes	2020-04-13	EPI_ISL_454125
Portugal/PT0672/2020	Europe / Portugal		2020-04-13	EPI_ISL_510931
Portugal/PT0951/2020	Europe / Portugal		2020-04-13	EPI_ISL_511501
Portugal/PT0952/2020	Europe / Portugal		2020-04-13	EPI_ISL_511502
Portugal/PT0389a/2020	Europe / Portugal		2020-04-14	EPI_ISL_454105
Portugal/PT0390a/2020	Europe / Portugal		2020-04-14	EPI_ISL_454108
Portugal/PT0391a/2020	Europe / Portugal		2020-04-14	EPI_ISL_454110
Portugal/PT0392/2020	Europe / Portugal	yes	2020-04-14	EPI_ISL_454111
Portugal/PT0393/2020	Europe / Portugal	yes	2020-04-14	EPI_ISL_454112
Portugal/PT0394/2020	Europe / Portugal	yes	2020-04-14	EPI_ISL_454113
Portugal/PT0546/2020	Europe / Portugal		2020-04-14	EPI_ISL_454270
Portugal/PT0567/2020	Europe / Portugal		2020-04-14	EPI_ISL_454290
Portugal/PT0569/2020	Europe / Portugal		2020-04-14	EPI_ISL_454292
Portugal/PT0570/2020	Europe / Portugal		2020-04-14	EPI_ISL_454293
Portugal/PT0953/2020	Europe / Portugal		2020-04-14	EPI_ISL_511503
Portugal/PT0688/2020	Europe / Portugal		2020-04-15	EPI_ISL_510947
Portugal/PT0689/2020	Europe / Portugal		2020-04-15	EPI_ISL_510948
Portugal/PT0283a/2020	Europe / Portugal		2020-04-16	EPI_ISL_453997
Portugal/PT0454/2020	Europe / Portugal		2020-04-17	EPI_ISL_454178

Portugal/PT0455/2020	Europe / Portugal		2020-04-17	EPI_ISL_454179
Portugal/PT0459/2020	Europe / Portugal		2020-04-17	EPI_ISL_454183
Portugal/PT0460/2020	Europe / Portugal		2020-04-17	EPI_ISL_454184
Portugal/PT0461/2020	Europe / Portugal		2020-04-17	EPI_ISL_454185
Portugal/PT0462/2020	Europe / Portugal		2020-04-17	EPI_ISL_454186
Portugal/PT0463/2020	Europe / Portugal		2020-04-17	EPI_ISL_454187
Portugal/PT0464/2020	Europe / Portugal		2020-04-17	EPI_ISL_454188
Portugal/PT0674/2020	Europe / Portugal		2020-04-17	EPI_ISL_510933
Portugal/PT0690/2020	Europe / Portugal		2020-04-17	EPI_ISL_510949
Portugal/PT0954/2020	Europe / Portugal		2020-04-17	EPI_ISL_582515
Portugal/PT0958/2020	Europe / Portugal		2020-04-17	EPI_ISL_511507
Portugal/PT0395a/2020	Europe / Portugal	yes	2020-04-18	EPI_ISL_454114
Portugal/PT0396a/2020	Europe / Portugal		2020-04-18	EPI_ISL_454117
Portugal/PT0456/2020	Europe / Portugal		2020-04-18	EPI_ISL_454180
Portugal/PT0457/2020	Europe / Portugal		2020-04-18	EPI_ISL_454181
Portugal/PT0465/2020	Europe / Portugal		2020-04-18	EPI_ISL_454189
Portugal/PT0466/2020	Europe / Portugal		2020-04-18	EPI_ISL_454190
Portugal/PT0467/2020	Europe / Portugal		2020-04-18	EPI_ISL_454191
Portugal/PT0468/2020	Europe / Portugal		2020-04-18	EPI_ISL_454192
Portugal/PT0469/2020	Europe / Portugal		2020-04-18	EPI_ISL_454193
Portugal/PT0470a/2020	Europe / Portugal		2020-04-18	EPI_ISL_454194
Portugal/PT0471/2020	Europe / Portugal		2020-04-18	EPI_ISL_454195
Portugal/PT0472/2020	Europe / Portugal		2020-04-18	EPI_ISL_454196
Portugal/PT0397a/2020	Europe / Portugal	yes	2020-04-19	EPI_ISL_454119
Portugal/PT0398/2020	Europe / Portugal	yes	2020-04-19	EPI_ISL_454121
Portugal/PT0399a/2020	Europe / Portugal		2020-04-19	EPI_ISL_454122
Portugal/PT0458/2020	Europe / Portugal		2020-04-19	EPI_ISL_454182
Portugal/PT0691/2020	Europe / Portugal		2020-04-19	EPI_ISL_510950
Portugal/PT0955/2020	Europe / Portugal		2020-04-19	EPI_ISL_511504
Portugal/PT0957/2020	Europe / Portugal		2020-04-19	EPI_ISL_511506
Portugal/PT0284/2020	Europe / Portugal		2020-04-20	EPI_ISL_453999
Portugal/PT0400/2020	Europe / Portugal	yes	2020-04-20	EPI_ISL_454124
Portugal/PT0556/2020	Europe / Portugal		2020-04-20	EPI_ISL_454279
Portugal/PT0557/2020	Europe / Portugal		2020-04-20	EPI_ISL_454280
Portugal/PT0956/2020	Europe / Portugal		2020-04-20	EPI_ISL_511505
Portugal/PT0558/2020	Europe / Portugal		2020-04-21	EPI_ISL_454281
Portugal/PT0402/2020	Europe / Portugal	yes	2020-04-22	EPI_ISL_454126
Portugal/PT0559/2020	Europe / Portugal		2020-04-22	EPI_ISL_454282
Portugal/PT0560/2020	Europe / Portugal		2020-04-22	EPI_ISL_454283
Portugal/PT0692/2020	Europe / Portugal		2020-04-23	EPI_ISL_510951
Portugal/PT0473/2020	Europe / Portugal		2020-04-24	EPI_ISL_454197
Portugal/PT0474/2020	Europe / Portugal		2020-04-24	EPI_ISL_454198
Portugal/PT0475/2020	Europe / Portugal		2020-04-24	EPI_ISL_454199
Portugal/PT0476/2020	Europe / Portugal		2020-04-24	EPI_ISL_454200
Portugal/PT0581/2020	Europe / Portugal		2020-04-24	EPI_ISL_454304
Portugal/PT0880/2020	Europe / Portugal		2020-04-24	EPI_ISL_511139
Portugal/PT1150/2020	Europe / Portugal		2020-04-24	EPI_ISL_511697
Portugal/PT0675/2020	Europe / Portugal		2020-04-25	EPI_ISL_510934
Portugal/PT0693/2020	Europe / Portugal		2020-04-25	EPI_ISL_510952
Portugal/PT0925/2020	Europe / Portugal		2020-04-25	EPI_ISL_511182
Portugal/PT0926/2020	Europe / Portugal		2020-04-25	EPI_ISL_511183
Portugal/PT1151/2020	Europe / Portugal		2020-04-25	EPI_ISL_511698
Portugal/PT1152/2020	Europe / Portugal		2020-04-25	EPI_ISL_511699
Portugal/PT1153/2020	Europe / Portugal		2020-04-25	EPI_ISL_511700
Portugal/PT1154/2020	Europe / Portugal		2020-04-25	EPI_ISL_511701
Portugal/PT1155/2020	Europe / Portugal		2020-04-25	EPI_ISL_511702
Portugal/PT1156/2020	Europe / Portugal		2020-04-25	EPI_ISL_511703
Portugal/PT0477/2020	Europe / Portugal		2020-04-26	EPI_ISL_454201
Portugal/PT0881/2020	Europe / Portugal		2020-04-26	EPI_ISL_511140
Portugal/PT0927/2020	Europe / Portugal		2020-04-26	EPI_ISL_511184
Portugal/PT1157/2020	Europe / Portugal		2020-04-26	EPI_ISL_511704
Portugal/PT0489/2020	Europe / Portugal		2020-04-27	EPI_ISL_454213
Portugal/PT0561/2020	Europe / Portugal		2020-04-27	EPI_ISL_454284
Portugal/PT0562/2020	Europe / Portugal	yes	2020-04-27	EPI_ISL_454285
Portugal/PT0490/2020	Europe / Portugal		2020-04-28	EPI_ISL_454214

Portugal/PT0844/2020	Europe / Portugal		2020-04-28	EPI_ISL_511103
Portugal/PT0491/2020	Europe / Portugal		2020-04-29	EPI_ISL_454215
Portugal/PT0492/2020	Europe / Portugal		2020-04-29	EPI_ISL_454216
Portugal/PT0493/2020	Europe / Portugal		2020-04-29	EPI_ISL_454217
Portugal/PT0494/2020	Europe / Portugal		2020-04-29	EPI_ISL_454218
Portugal/PT0959/2020	Europe / Portugal		2020-04-29	EPI_ISL_511508
Portugal/PT0495/2020	Europe / Portugal		2020-04-30	EPI_ISL_454219
Portugal/PT0496/2020	Europe / Portugal		2020-04-30	EPI_ISL_454220
Portugal/PT0847/2020	Europe / Portugal		2020-04-30	EPI_ISL_511106
Portugal/PT0882/2020	Europe / Portugal		2020-04-30	EPI_ISL_511141
Portugal/PT0497/2020	Europe / Portugal		2020-05-01	EPI_ISL_454221
Portugal/PT0548/2020	Europe / Portugal	yes	2020-05-02	EPI_ISL_454272
Portugal/PT0563/2020	Europe / Portugal	yes	2020-05-02	EPI_ISL_454286
Portugal/PT0593/2020	Europe / Portugal		2020-05-04	EPI_ISL_454316
Portugal/PT0550/2020	Europe / Portugal	yes	2020-05-05	EPI_ISL_454273
Portugal/PT0771/2020	Europe / Portugal		2020-05-05	EPI_ISL_511030
Portugal/PT0846/2020	Europe / Portugal		2020-05-05	EPI_ISL_511105
Portugal/PT0884/2020	Europe / Portugal		2020-05-05	EPI_ISL_511142
Portugal/PT0845/2020	Europe / Portugal		2020-05-06	EPI_ISL_511104
Portugal/PT0885/2020	Europe / Portugal		2020-05-06	EPI_ISL_511143
Portugal/PT1136/2020	Europe / Portugal		2020-05-06	EPI_ISL_511683
Portugal/PT1137/2020	Europe / Portugal		2020-05-06	EPI_ISL_511684
Portugal/PT0772/2020	Europe / Portugal		2020-05-17	EPI_ISL_511031
Portugal/PT1031/2020	Europe / Portugal		2020-05-21	EPI_ISL_511578
Portugal/PT1032a/2020	Europe / Portugal		2020-06-05	EPI_ISL_511579
Portugal/PT1033/2020	Europe / Portugal		2020-06-06	EPI_ISL_511580

Table S2. List of SARS-CoV-2 Spike amino acid sequences with mutations in the 839 site available at GISAID, as of July 23rd, 2020.

virus ID	Mutation in Spike amino acid D839	Spike D614G background	Nexstrain clade ^a	GISAID Accession ID ^b	date of collection	continent	country	region
Italy/PV-5314-N/2020	D839Y	G614	20A	EPI_ISL_451307	2020-02-21	Europe	Italy	Lombardy
Netherlands/Diemen_1363454/2020	D839E	G614	20B	EPI_ISL_413570	2020-02-28	Europe	Netherlands	Netherlands
England/20099107406/2020	D839Y	G614	20A	EPI_ISL_415131	2020-02-29	Europe	United Kingdom	England
England/20102000506/2020	D839Y	G614	20A	EPI_ISL_415147	2020-03-01	Europe	United Kingdom	England
England/201061455/2020	D839Y	G614	20A	EPI_ISL_464413	2020-03-01	Europe	United Kingdom	England
England/CAMB-847A5/2020	D839Y	G614	20A	EPI_ISL_440513	2020-03-01	Europe	United Kingdom	England
Iceland/14/2020	D839Y	G614	20A	EPI_ISL_417736	2020-03-01	Europe	Iceland	Reykjavik
England/20102132304/2020	D839Y	G614	20A	EPI_ISL_464290	2020-03-02	Europe	United Kingdom	England
England/20104009002/2020	D839Y	G614	20A	EPI_ISL_417233	2020-03-02	Europe	United Kingdom	England
Iceland/20/2020	D839Y	G614	20A	EPI_ISL_417695	2020-03-02	Europe	Iceland	Reykjavik
England/20108003302/2020	D839Y	G614	20A	EPI_ISL_417248	2020-03-03	Europe	United Kingdom	England
England/20108004803/2020	D839Y	G614	20A	EPI_ISL_417251	2020-03-03	Europe	United Kingdom	England
England/201080050/2020	D839Y	G614	20A	EPI_ISL_464442	2020-03-03	Europe	United Kingdom	England
England/BRIS-12177C/2020	D839Y	G614	20A	EPI_ISL_440256	2020-03-03	Europe	United Kingdom	England
England/BRIS-12178B/2020	D839Y	G614	20A	EPI_ISL_440212	2020-03-03	Europe	United Kingdom	England
England/BRIS-12F5A1/2020	D839Y	G614	20A	EPI_ISL_488219	2020-03-03	Europe	United Kingdom	England
England/201060017/2020	D839Y	G614	20A	EPI_ISL_464362	2020-03-04	Europe	United Kingdom	England
England/201080076/2020	D839Y	G614	20A	EPI_ISL_464459	2020-03-04	Europe	United Kingdom	England
England/20110000706/2020	D839Y	G614	20A	EPI_ISL_417302	2020-03-05	Europe	United Kingdom	England
England/20110000606/2020	D839Y	G614	20A	EPI_ISL_417301	2020-03-06	Europe	United Kingdom	England
England/20110000906/2020	D839Y	G614	20A	EPI_ISL_417304	2020-03-06	Europe	United Kingdom	England
England/BRIS-12F617/2020	D839Y	G614	20A	EPI_ISL_488253	2020-03-06	Europe	United Kingdom	England
England/20109007304/2020	D839Y	G614	20A	EPI_ISL_464527	2020-03-07	Europe	United Kingdom	England
England/201090513/2020	D839Y	G614	20A	EPI_ISL_464577	2020-03-07	Europe	United Kingdom	England
Iceland/69/2020	D839Y	G614	20A	EPI_ISL_417749	2020-03-08	Europe	Iceland	Reykjavik
England/201120242/2020	D839Y	G614	20A	EPI_ISL_464672	2020-03-09	Europe	United Kingdom	England
England/201140338/2020	D839Y	G614	20A	EPI_ISL_464724	2020-03-09	Europe	United Kingdom	England
England/BRIS-12F750/2020	D839Y	G614	20A	EPI_ISL_488349	2020-03-09	Europe	United Kingdom	England

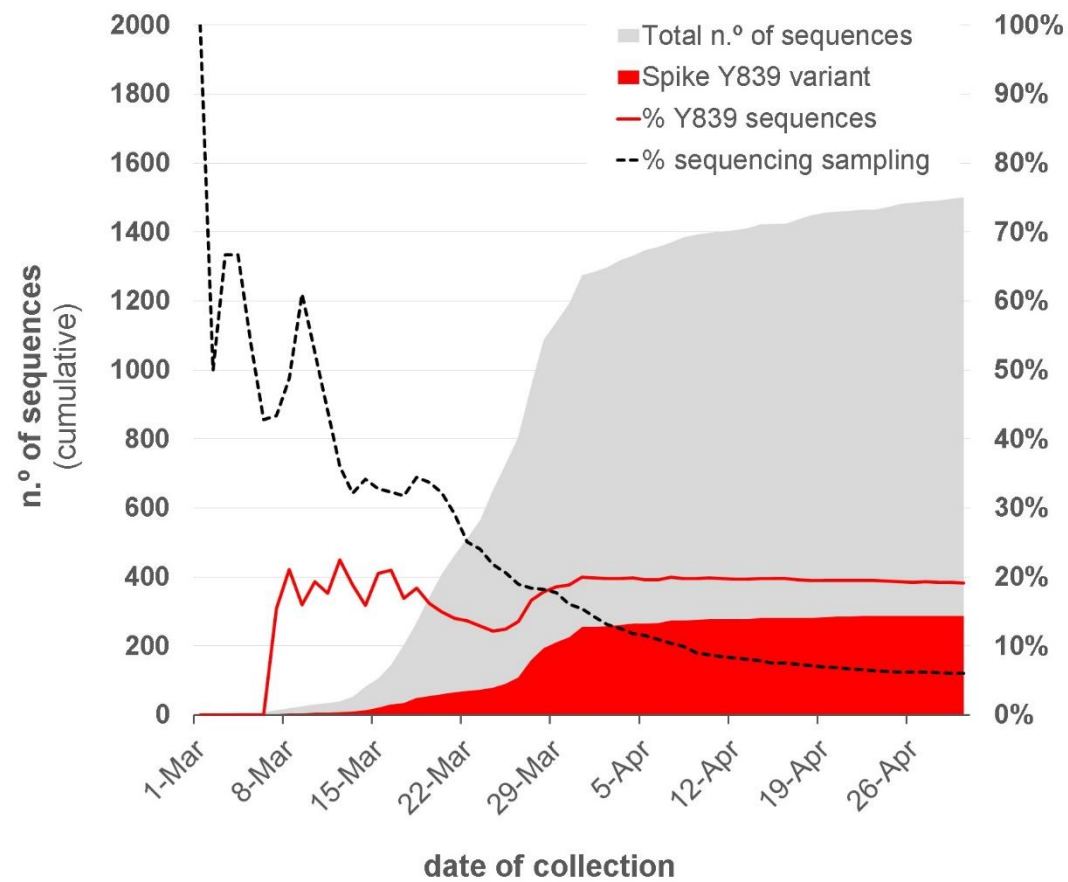
Iceland/103/2020	D839Y	G614	20A	EPI_ISL_417698	2020-03-10	Europe	Iceland	Reykjavik
England/201140062/2020	D839Y	G614	20A	EPI_ISL_464713	2020-03-11	Europe	United Kingdom	England
England/20119002104/2020	D839Y	G614	20A	EPI_ISL_465007	2020-03-12	Europe	United Kingdom	England
England/CAMB-75516/2020	D839Y	G614	20A	EPI_ISL_425401	2020-03-13	Europe	United Kingdom	England
Georgia/Tb/2020	D839Y	G614	20A	EPI_ISL_416482	2020-03-13	Asia	Georgia	Tbilisi
Netherlands/ZuidHolland_44/2020	D839Y	G614	20A	EPI_ISL_422916	2020-03-19	Europe	Netherlands	South Holland
Netherlands/ZuidHolland_45/2020	D839Y	G614	20A	EPI_ISL_422917	2020-03-19	Europe	Netherlands	South Holland
Netherlands/ZuidHolland_52/2020	D839Y	G614	20A	EPI_ISL_422920	2020-03-19	Europe	Netherlands	South Holland
Poland/1105973/2020	D839Y	G614	20A	EPI_ISL_428234	2020-03-19	Europe	Poland	Pomorskie
NewZealand/20VR1278/2020	D839Y	G614	20A	EPI_ISL_456203	2020-03-20	Oceania	New Zealand	Auckland
Netherlands/NA_165/2020	D839Y	G614	20A	EPI_ISL_422700	2020-03-21	Europe	Netherlands	Netherlands
Netherlands/NA_173/2020	D839Y	G614	20A	EPI_ISL_422708	2020-03-22	Europe	Netherlands	Netherlands
Netherlands/Utrecht_10022/2020	D839Y	G614	20A	EPI_ISL_454771	2020-03-23	Europe	Netherlands	Utrecht
England/NORT-281D2C/2020	D839G	D614	19A	EPI_ISL_478483	2020-03-24	Europe	United Kingdom	England
Netherlands/ZuidHolland_73/2020	D839Y	G614	20A	EPI_ISL_422941	2020-03-24	Europe	Netherlands	South Holland
Austria/CeMM0397/2020	D839Y	G614	20A	EPI_ISL_475834	2020-03-25	Europe	Austria	Austria
Wales/PHWC-25EDE/2020	D839N	D614	19A	EPI_ISL_422317	2020-03-25	Europe	United Kingdom	Wales
Switzerland/100799/2020	D839Y	G614	20A	EPI_ISL_451749	2020-03-25	Europe	Switzerland	Uri
Switzerland/100804/2020	D839Y	G614	20A	EPI_ISL_451754	2020-03-25	Europe	Switzerland	Uri
USA/TX-HMH0273/2020	D839Y	G614	20A	EPI_ISL_434901	2020-03-26	America	USA	Texas
Switzerland/110014_159_F6/2020	D839Y	G614	20A	EPI_ISL_486454	2020-04-01	Europe	Switzerland	Uri
Netherlands/NA_308/2020	D839Y	G614	20A	EPI_ISL_422617	2020-04-02	Europe	Netherlands	Netherlands
NewZealand/20VR2058/2020	D839Y	G614	20A	EPI_ISL_456352	2020-04-05	Oceania	New Zealand	Canterbury
NewZealand/20VR2065/2020	D839Y	G614	20A	EPI_ISL_456356	2020-04-06	Oceania	New Zealand	Canterbury
NewZealand/20VR2067/2020	D839Y	G614	20A	EPI_ISL_456358	2020-04-06	Oceania	New Zealand	Canterbury
England/EXET-136DAF/2020	D839Y	G614	20A	EPI_ISL_471924	2020-04-07	Europe	United Kingdom	England
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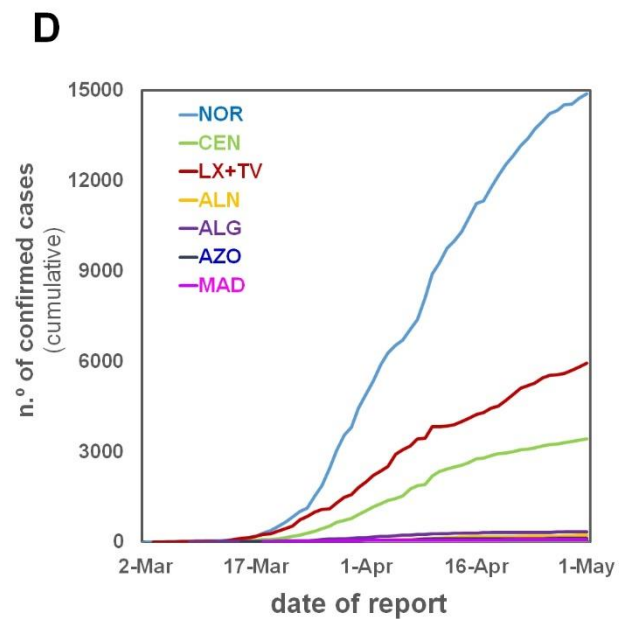
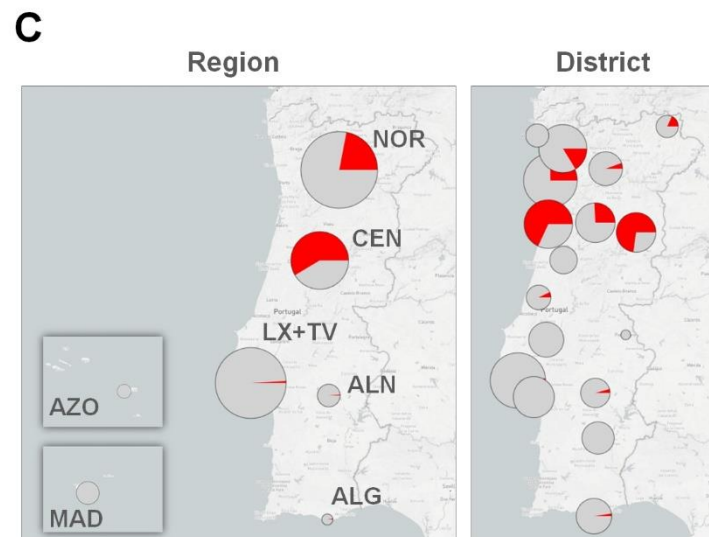
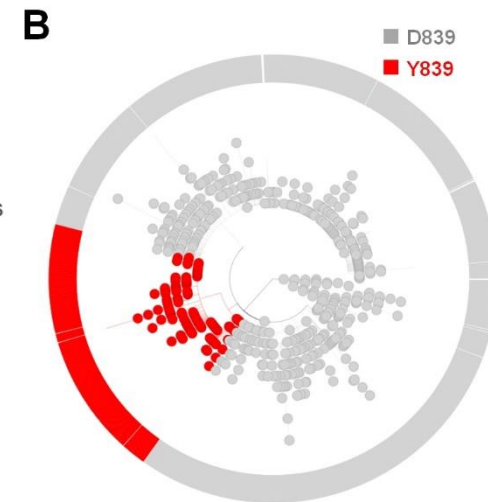
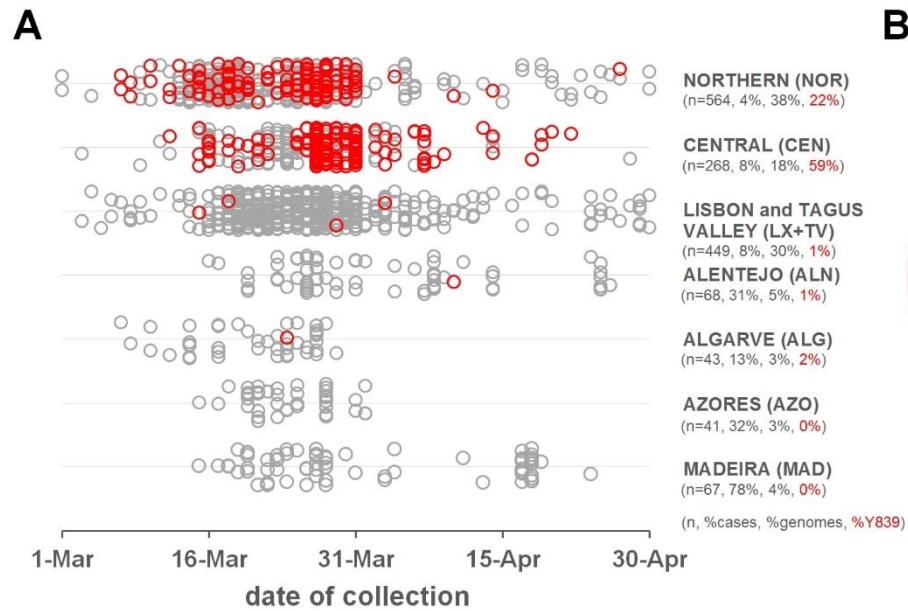
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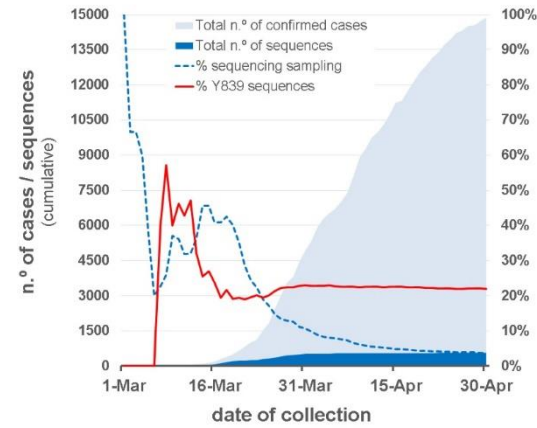
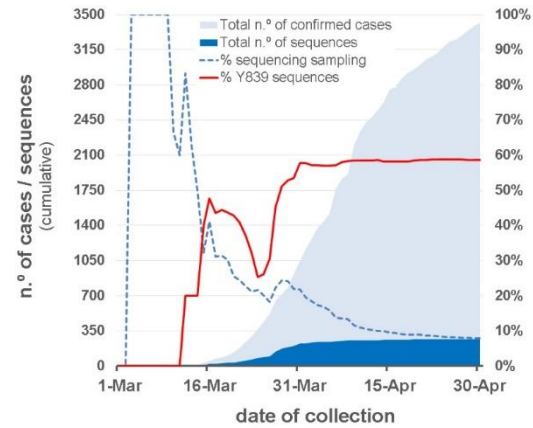
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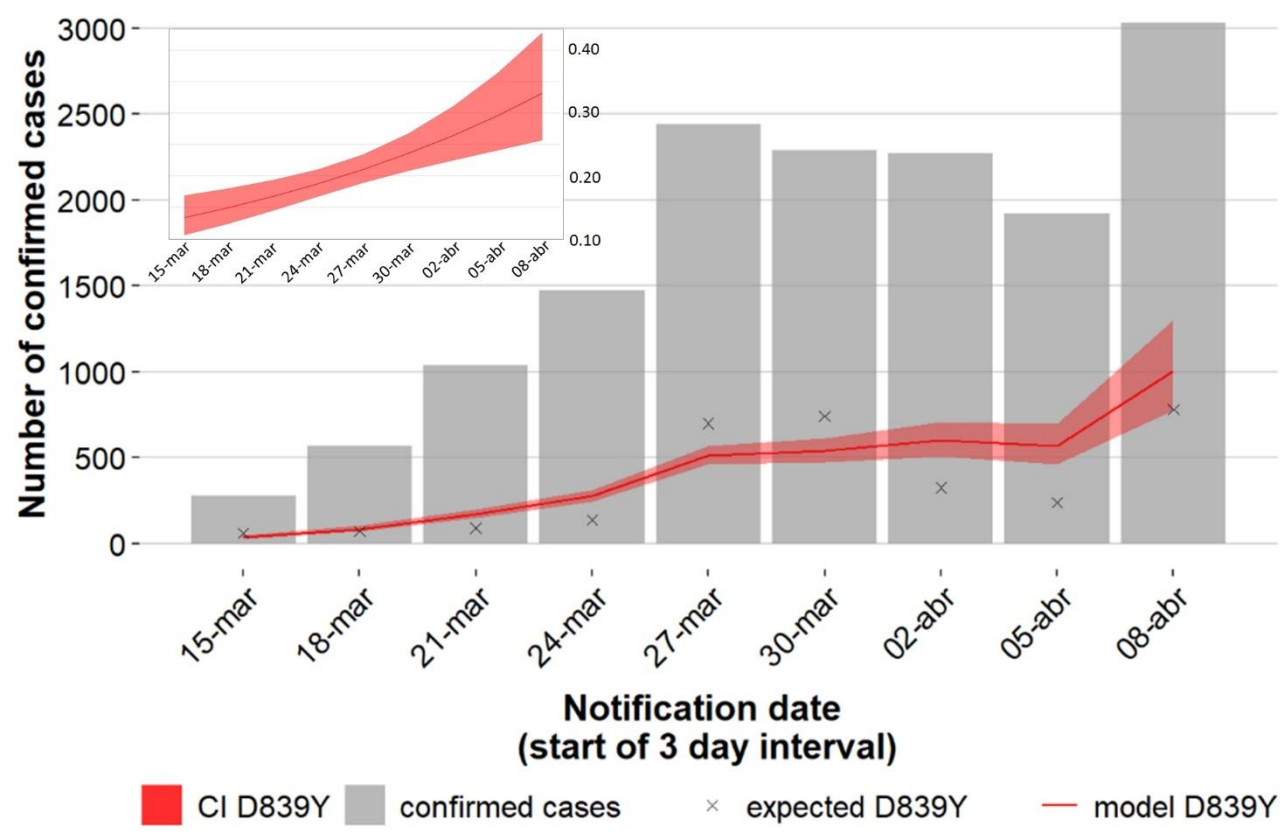
^a Nextstrain clade was determined using Nextclade online tool (<https://clades.nextstrain.org/>).

^b Acknowledgement regarding sequences from GISAID's EpiFlu™ Database: We acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's Database on which this research is based.





A**B**



ACCEPTED

