

Review and Commentary on:
‘Molecular epidemiology of SARS-CoV-2 in Greece reveals low rates of onward virus transmission after lifting of travel restrictions based on risk assessment during summer’ *

Harris Georgiou¹

¹University of Piraeus[†] – Data Science Lab[‡]
hgeorgiou@unipi.gr

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Abstract

This is a short review and commentary on a paper that was recently published, regarding the effects of opening the borders during the summer of 2020 in Greece and how it allegedly did not result in significant SARS-CoV-2 imported infections from travellers. In this commentary, this thesis is proven unsupported by sufficient evidence, as the study presents several deficiencies and biases regarding the statistical context and the proof of the core hypothesis, as described herein.

Keywords — SARS-CoV-2, COVID-19, epidemics, screening methods, Greece

1 Introduction

On February 2nd, 2020, a paper [1] was published by a group of epidemiologists and other scientists currently working with the advisory group of the Greek government regarding the management and policy planning for the national SARS-CoV-2 epidemic for the last year. In their study, the authors claim that, according to phylogenetic analytics on virus strains detected in Greece in three time pe-

riods, the lifting of strict travel restrictions during the summer of 2020 did not result in a significant inflow of infections among the travellers. They further justify this result by the implementation of targeted border checks with the assistance of AI-based decision support, which allegedly mitigated the high rate of arrivals and the very low ratio of actual tests conducted.

According to the review described in this commentary, the aforementioned study, particularly the statistical and inference aspect of the experimental protocol, presents several deficiencies and drawbacks, which undermine the validity of the authors’ core thesis. More specifically, these deficiencies can be grouped around:

- temporal inconsistency / bias
- spatial inconsistency / bias
- volume of sample data
- virus strain lineage evolution profile
- correlation between arrivals and infections
- insufficient interpretation of findings
- additional contradictive information

Each of these items is cited and discussed in detail in the following sections.

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[†]<http://www.unipi.gr> – Address: 80 Karaoli & Dimitriou str., GR-18534 Greece.

[‡]<http://datastories.org>

2 Detailed comments

2.1 Temporal inconsistency/bias

In this part, several issues are described related to temporal bias and statistical incoherence of the selected sample data with regard to the main hypothesis of the study.

► According to the authors (pg.3):

‘The proportion of imported strains was 41%, 11.5%, and 8.8% during the three periods of sampling, namely, March (no travel restrictions), April to June (strict travel restrictions), and July to September (lifting of travel restrictions based on a thorough risk assessment), respectively. These findings reveal low levels of onward transmission from imported cases during summer (...)’

This is the core thesis of the authors in their study. Comparison between the second to the third time period may be valid, since the epidemic was onset at the national level by that time. However, it is not statistically valid regarding the first period, when the stationarity of the underlying dynamic system is highly volatile and epidemic characteristics are very different, especially the lockdown policies employed in Greece internally and travel-related. Furthermore, these numbers are based on numerous assumptions and reasoning without sufficient experimental and statistical support, as it is explained in the next sections.

► According to the authors (pg.4):

‘...between the lifting of the first measures in May and this second wave, the number of cases remained relatively low even after travel restrictions were lifted at the beginning of July 2020.’

This assertion is not accurate. The epidemic data for Greece¹, presented in Fig. 1, show that the confirmed cases were rising significantly after the third week of July and continued to do so at a lower rate up to the end of August, with a small deflation at the beginning of September and then rise steadily until mid-October, when the subsequent wave of the epidemic was in full surge.

Additionally, the national data show that, although the screening tests (see Fig. 2 and Fig. 3) in July were doubled compared to June, they were still far fewer than the monthly tests con-

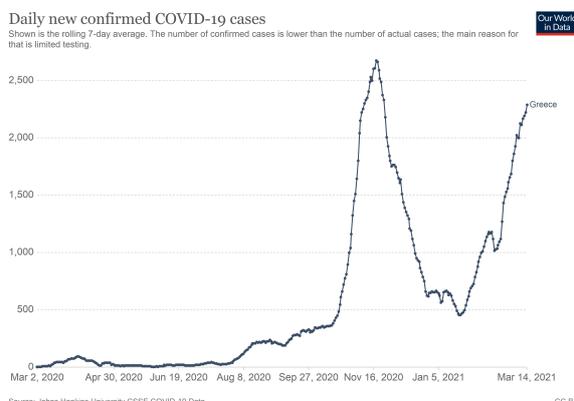


Figure 1: Greece, 2020: Confirmed COVID-19 daily cases.

ducted in August and September, which in turn were again fewer than those conducted in October. Hence, it is almost certain that there is some underrepresentation of the true prevalence of the virus in the general population for most of the summer period and well within September. This is confirmed by the fact that the positive rate in the screening tests gradually rises from about 0.5% at the beginning of summer to 1.43% in August, 2.30% in September, 4.43% in October, etc.

► According to the authors (pg.5):

‘Our sampling comprised three time periods: between February 29 and March 31; between April 1 and June 30; and between July 1 and September 29, 2020.’

► According to the authors (pg.8):

‘As mentioned in materials and methods section, sampling process comprised three time periods. The samples included in the study were as follows: 156 of 1,565 diagnosed cases (10%) for the first period, 101 of 1,873 cases (5.4%) for the second period, and 132 of 15,869 cases (0.8%) for the third period. The lower proportion for the third period was due to the number of tests performed increasing gradually with time (i.e., the average number of tests per month was approximately 10x higher in the third versus the first period), suggesting that the last period was not underrepresented in our sample.’

The data sampling with regard to the three different time periods seems typically adequate for proper statistical representation. However, for the third period the available cases are a full order of

¹Our World In Data – Greece: Coronavirus Pandemic Country Profile <https://ourworldindata.org/coronavirus/country/greece>

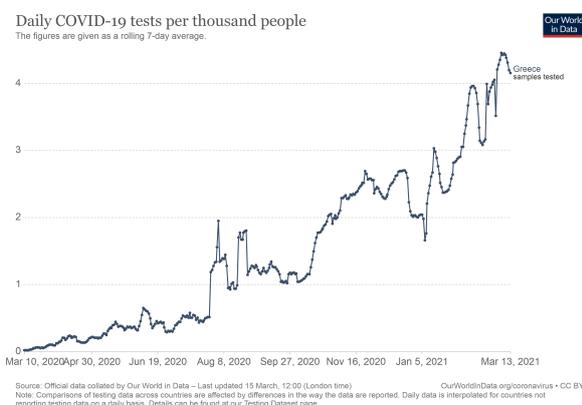


Figure 2: Greece, 2020: COVID-19 daily tests per thousand people.

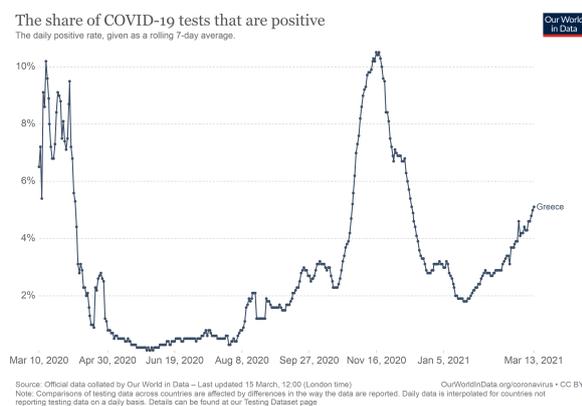


Figure 3: Greece, 2020: Positive rate in daily COVID-19 daily tests.

magnitude larger in volume than for the other two, hence taking the same subsampling size essentially degrades the quality and significance of this group by a large margin. Taking into account that the samples correspond to three different geographical areas with high bias (90.7% from Attica), the 132 samples for the third period poses questions about the statistical significance of this entire subset of data.

2.2 Spatial inconsistency/bias

In this part, several issues are described related to spatial bias and statistical incoherence of the selected sample data with regard to the main hypoth-

esis of the study.

► According to the authors (pg.4):

‘However, by the end of October 2020 the country experienced rapid increases in the number of SARS-CoV-2 cases in the metropolitan area of Thessaloniki and other areas of Northern Greece.’

► According to the authors (pg.5):

‘The SARS-CoV-2 samples analyzed in the context of the current study were collected from February 29 to September 19, 2020 in the Attica, Larisa and Thrace regions (...) SARS-CoV-2 samples obtained at border control areas from travelers arriving in Greece were excluded from the analysis.’

The spatial relevance of the sample data is invalid. The summer period in Greece and the inflow of tourist from other countries is almost entirely associated with Crete, the Aegean islands and the Ionian islands. Larissa and Thrace have almost nothing to do with this context. Additionally, any travellers arriving to Greece via Athens are in very limited contact with the local population, since they are en route to other destinations via the international airport (sometimes with transit flights, never leaving the terminal areas) or directly to the port of Piraeus to continue their journey by ship. Hence, spatial relevance of the data sample is only for Attica (marginally), only for a few hours per-case and only in the main transit hubs, namely the airport and the port.

Regarding the subsequent surge of the epidemic, although Thessaloniki is identified as the major ‘hotzone’ during October, it was excluded from the sample data. Other regions that also present high mobility of travellers are Epirus and Western Macedonia, where under normal conditions borders are crossed by thousands of people to and from Greece to other Balkan countries. These regions were also excluded from the study. Furthermore, the authors clearly state that all samples taken at the border control areas were excluded from this study. There is no explanation or reasoning to support these choices, which in fact undermine the core thesis of the authors’ paper.

2.3 Volume of sample data

In this part, several issues are described related to the volume and statistical significance of the selected sample data with regard to the main hypothesis of the study.

► According to the authors (pg.7-8):

‘Our study data comprised of 389 unique full-genome SARS-CoV-2 sequences, of which 280 were newly generated and 109 were available on the GISAID database, collected in Attica until December 1, 2020 [35]. The vast majority of our samples had been collected in Attica (N=353, 90.7%). To investigate the patterns of SARS-CoV-2 infection in the areas of Northeast Greece and Thessaly, where virus surges were reported in March and May, respectively, we analysed 17 samples drawn from Alexandroupoli, Kavala, Komotini and Xanthi in Northeast Greece and 13 samples from the Nea Smirni area in Larissa, Thessaly. A few samples (N=4) analyzed as part of routine diagnostic testing in Attica were also available from 3 Aegean islands.’

These numbers confirm that the data sample has little to no real spatial relevance to the main destinations of possible inflow of infections from tourists. There are only four samples from three different Aegean islands, i.e., about 1% (4/389) that may correspond to tourist destinations during that time period, hence statistically insignificant for any hypothesis testing.

► According to the authors (pg.9):

‘Given that our sample pool corresponds to 10% of the diagnosed cases and, also, that the actual number of SARS-CoV-2 infections should be severely underdiagnosed, the different lineages introduced to Greece should be higher than our estimation.’

The authors clearly state that their sample pool is too limited to ensure that it fully represents the statistical prevalence of all virus strains that may have been introduced to Greece from foreign travellers during these time periods. Again, this choice is stated with no explanation or reasoning to support it, which in fact undermines the core thesis of the authors’ paper.

2.4 Virus strain lineage / evolution profile

In this part, several issues are described related to the statistical aspects and hypothesis testing of the main (molecular epidemiology) experimental protocol with regard to the main hypothesis of the study.

► According to the authors (pg.7):

*‘The viral migration events were quantified between the different geographic areas/countries by character reconstruction using the criterion of parsimony as implemented in PAUP*4.0 [29]. We assessed whether the inferred migration events (imported or local infections) were different from those expected by chance (panmixis).’*

► According to the authors (pg.8-9):

‘The results of the classification of viral sequences into lineages, as estimated using the pangolin program, are shown in Table 1. The most frequent lineages were B.1.1 (European lineage; 40.6%), B.1.1.152 (Russian lineage; 19.5%), B1.1.38 (the UK lineage; 11.8%), (...) The largest cluster consisted of 37 (16.7%; first period), 72 (32.6%; second period) and 112 (50.7%; third period) sequences collected during the respective sampling periods (Figure 2C).’

There is an interesting statistical trend in the data of Table 1. It is clear that strains B.1.1 (‘European’), B.1.1.38 (‘UK’) and B.1.1.152 (‘Russian’) were the three most prevalent strains of the virus in Greece during the time periods examined in the study, as well as in the largest cluster of 221 sequences. It is also clear that their prevalence increases significantly in every successive time period investigated, much higher in terms of impact than any other virus strain in this table. In fact, while the ratio of the two strains B.1.1.38 and B.1.1.152 grouped together against the B.1.1 starts a 1:6.23 at the first period, it goes up to 1:1.39 at the second period and ends up at 2.70:1 at the third period, i.e., almost a 17-fold increase. These numbers indicate a clear shift in the main composition of the virus circulating in the general population, as roughly the same proportions are found in the largest cluster of sequences.

According to the PANGO lineages² of the SARS-CoV-2 strains, B.1.1.38 is highly correlated geographically to the UK (see Fig. 4) and appeared as early as 24/3/2020, but its global prevalence deflated before the summer period (see Fig. 5). Similarly, B.1.1.152 is highly correlated to the USA, UK and Russia (see Fig. 6) and appeared as early as 27/3/2020, but its global prevalence inflated after the main summer period (see Fig. 7). None of these strains appear as prevalent in the first period, i.e.,

²PANGO lineages database, B1.1.x – https://cov-lineages.org/lineages/lineage_B.1.1.html

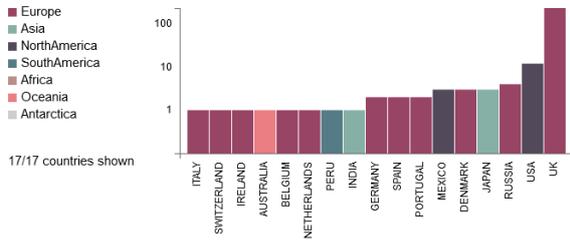


Figure 4: PANGO lineages: B.1.1.38 ('UK') spatial prevalence.

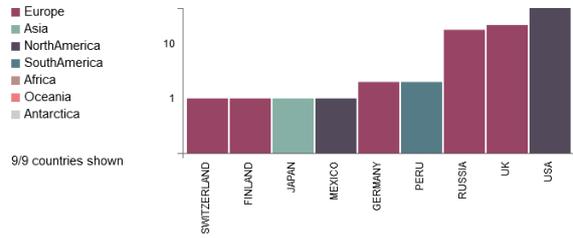


Figure 6: PANGO lineages: B.1.1.152 ('Russian') spatial prevalence.

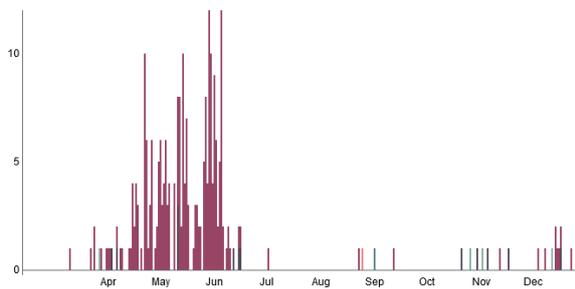


Figure 5: PANGO lineages: B.1.1.38 ('UK') temporal prevalence.

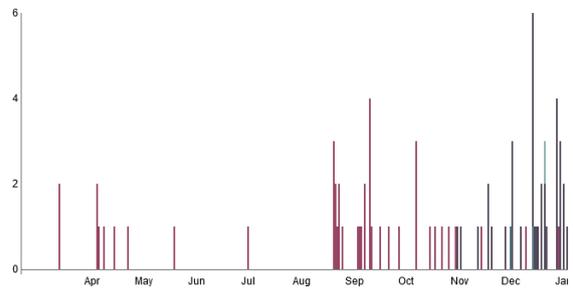


Figure 7: PANGO lineages: B.1.1.152 ('Russian') temporal prevalence.

up to the end of March 2020 when no travel restrictions were in place, but both become more evident even in the second period of April to June, when strict travel restrictions were in place.

If the authors' core thesis is valid, then the evolution profile of these specific strains should be evident in other countries too, in the same periods and with the same travel restrictions. On the contrary, countries in Europe with much more relaxed travel restrictions during and after the summer should exhibit a quite different evolution profile and strains prevalence. In other words, the authors should present a proper statistical definition of the exact hypothesis to be tested, with at least one control case (country) as baseline and Greece as the testing case. Instead, the authors present only the evolution profile for Greece compared to the panmixis template (purely random) and claim that this evolution profile provides sufficient evidence that no significant inflow of infected cases was realized during the summer. Without a proper control, it is impossible to compare and statistically infer with sufficient support whether the B.1.1.38

or B.1.1.152 or any other virus strain were present in Greece during March, or introduced during the strict travel restrictions, or introduced after the lifting of the travel restrictions during summer. Particularly for the B.1.1.152 strain, it seems that it might have been present and spreading rapidly in the general population in Greece well within the summer, before its global prevalence was recorded from September and forward, at least according to some lineage databases (see Fig. 7).

2.5 Correlation between arrivals and infections

In this part, several issues are described related to the statistical correlation between arrivals and confirmed cases during the time frames of the sample data with regard to the main hypothesis of the study.

► According to the authors (p.12-13):

'Notably, the proportion of imported infections remained low after the lifting of restrictions on international travel implemented on July 1 in Greece

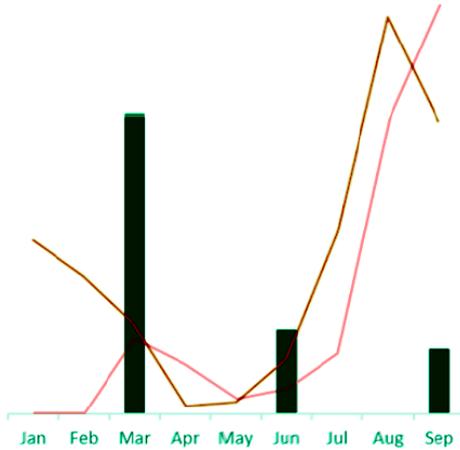


Figure 8: Overlay of the authors' Figure 3B and 3C, regarding the arrivals and confirmed cases per month, respectively.

(Figure 3B), and, although a virus surge was detected in August, it was not associated with an increased proportion of imported infections (Figure 3C).'

From their Figure 3 (pg.13), the statistical correlation between number of monthly arrivals and confirmed cases (red solid lines) is clear and self-explanatory, probably at 0.9 or above. Fig. 8 presents an overlay of the authors' Figure 3B and 3C, regarding the arrivals and confirmed cases per month, respectively, which confirm this high correlation level between the two. Hence, the corresponding estimations from the phylogeographic analysis (solid bars) should be either explained and supported with further statistical proof, or the authors should explain this controversy. Correlation does not imply causality, but any such counter-argument should be presented here in detail.

Additionally, although the daily confirmed cases for every country can be found in various sources, no citation is provided by the authors regarding the arrivals in Greece during these months in their Figure 3B.

► According to the authors (pg.14):

'No information about the origin of potential imported cases was available for the third period since virus screening was performed at the entry sites and the putative origins of the imported cases that remained undiagnosed was unknown.'

The authors clearly state that information that

associates imported cases of infections for the third time period (summer), both confirmed and undiagnosed, is not available or not taken into account in their study. There is no explanation why the study did not exploit information from the PLF data (see below), since this would enable some tracking of the origin of confirmed cases from abroad. Again, this choice is stated with no explanation or reasoning to support it, which in fact undermines the core thesis of the authors' paper.

2.6 Insufficient interpretation of findings

In this part, the interpretation by the authors of their findings is examined in terms of statistical significance and inference validity with regard to the main hypothesis of the study.

► According to the authors (pg.14):

'More importantly, we found that virus importation remained low and did not substantially contribute to SARS-CoV-2 onward transmission even after the lifting of travel restrictions.'

► According to the authors (pg.15):

'Our study suggests that the impact of travelers to SARS-CoV-2 local transmission in Greece was low during the summer. To our knowledge, this is one of the few molecular epidemiology studies showing that the lifting of travel restrictions after the first pandemic wave was not associated with onward transmission driven by imported SARS-CoV-2 cases.'

As explained above, this claim can not be supported by the experimental evidence presented in the authors' paper, since it has significant statistical drawbacks in terms of the temporal, spatial, volume and bias of the sample data used. If there are other similar molecular studies for other countries providing the same conclusions, they should be presented comparatively in the authors' study, applying compatible experimental protocol and evaluation metrics.

► According to the authors (pg.14-15):

'Since July 1, 2020, all incoming travelers, including Greek citizens, need to have completed a passenger locator form (PLF) 48 hours before entering Greece. Health screening procedures have been put in place at airports and other ports of entry, where targeted testing has been performed guided by an artificial intelligence algorithm termed

EVA. The algorithm combines information from previous tests performed at entry points in the country, as well as data obtained from the PLF creating an importation risk profile for each visitor according to country of travel origin. Health authorities can utilize this profile to determine border molecular testing prioritization, thus enhancing public health protection. Risk assessment for all countries was continuously performed daily and measures were modified accordingly (...)

The policies of using PLF as evidence is never used or exploited in the authors' paper, since they clearly state (see above) that such information was not available to them or decided to exclude it from their experimental study, i.e., as evidence for forming subgroups in the data sample and investigating the statistical significance w.r.t. the real discriminating value of PLF, e.g., country of origin.

Furthermore, the reference to the EVA algorithm is introduced without any citation to a corresponding methodology and peer-reviewed study that explains it. There is no explanation of this prioritization procedure and, obviously, the validity of the sample data in terms of statistical bias, e.g., under- or over-representation of specific subgroups. Hence, the claim that this policy, combined with PLF, explains the authors' findings in this paper can not be validated either. If the policy itself is not fully known, well-studied and confirmed as reliable and scientifically valid, it can not be asserted as an argument to support the findings of the study.

► According to the authors (pg.15):

'Notably, except for few islands (i.e., Paros, Mykonos), no virus surges were detected during the summer period in Greece and the effective reproductive number R remained around 1.1-1.2 during this period (National Public Health Organization; unpublished data).'

The authors refer to two specific Aegean island where there was indeed an epidemic surge during the summer. Nevertheless, these geographical areas are excluded from their study, making the sample data highly biased and non-representative for investigating possible correlation with tourists and tourist destinations.

It should also be noted that there was a continuous flow of new confirmed cases throughout the summer period in Crete and the major Aegean islands, as Fig. 9 and Fig. 10 clearly show. There is also solid evidence of severe under-reporting of

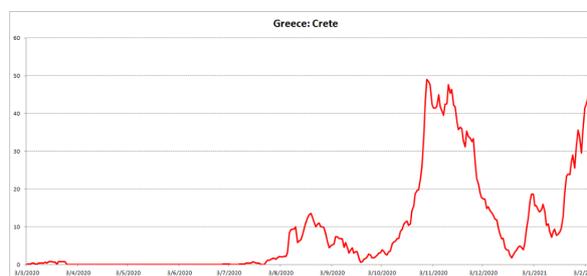


Figure 9: Greece, 2020: Confirmed COVID-19 cases for Crete.

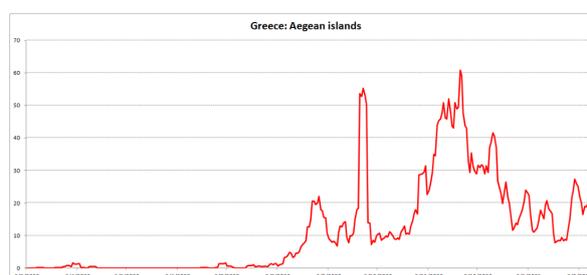


Figure 10: Greece, 2020: Confirmed COVID-19 cases for the major Aegean islands.

cases in the tourist regions³, as well as unreliable tracking of confirmed cases in the official data during the summer period. Foreigners were not tested upon exiting the country and there were also many transportations of suspicious cases to hospitals in the mainland for further testing and recording of positives away from their true origin.

More importantly, the authors refer to these findings and to 'unpublished data' from the NPHO, Greece. This obviously cancels any possibility of reproducing these experimental results, by any other researcher in Greece or elsewhere. Again, this choice is stated with no explanation or reasoning to support it, which in fact undermines the core thesis of the authors' paper.

► According to the authors (pg.15):

'...local clusters, that potentially have been ignited after the lifting of travel restrictions, may have remained undetected in our study.'

The authors clearly state that possible introductions of the virus of any strain to a small population, as in a tourist island, may have been missed in their experimental work.

³Source: <http://corfuvoice.gr>

Additionally, from the first wave of the epidemic in Greece it is now well-established that such small-scale events require at least two weeks of ‘incubation’ within the society before they become significant and detectable. For example, this is the time span between the first official confirmed case in Greece (Feb. 26, 2020) and the onset of the first country-wide restrictive measures about two weeks later, after the detection of virus surge in the local population. Hence, the ending date of the third period of the sample data in this study (Sept. 29, 2020) is consistent with the beginning of the major surge of the epidemic in mid-October. This does not mean that the surge resulted from confirmed infections at the end of September, but it certainly means that the experimental protocol implemented in this study does not rule out such a possibility - in order to do so, the sample data should include at least two more weeks beyond the end of September.

► According to the authors (pg.15):

‘...although our samples were not collected at tourist destinations, they were drawn from Attica, where almost 40% of the total Greek population resides, and which fuels tourism in these destinations during the summertime. Therefore, if new strains were associated with high levels of local transmission, we should have been able to detect them through our sampling.’

The authors confirm that tourist destinations were not included in their study, essentially making the sample data spatially irrelevant to the hypothesis testing, since neither the entry points of tourists were included.

Additionally, the claim that 40% of the total Greek population resides in Attica is irrelevant to what the core hypothesis tests, i.e., if new virus strains were introduced by foreign travellers en route to tourist destinations.

Furthermore, even in the case of internal tourism from Attica to other destinations in Greece, the time span of the third period does not cover the return of this indigenous population back to their residencies. This typically extends well after mid-September and, hence, any significant epidemic surges attributed to this movement would become evident only after the end of September (at least two weeks delay, see comment above), which was indeed the fact and how the subsequent surge evolved, starting from early/mid-October.

► According to the authors (pg.15):

‘Our study has several limitations. Our sampling was not representative and was not performed across Greece.’

The authors confirm that the sample data are not adequate to fully support their core hypothesis testing. Again, this choice is stated with no explanation or reasoning to support it, which in fact undermines the core thesis of the authors’ paper.

► According to the authors (pg.15-16):

‘Regarding the putative limitation of non-sampling from tourist destinations during the third phase, if SARS-CoV-2 was continuously transmitted from viral lineages imported during the summertime in Greece, we would be able to detect them in Attica residents, a large proportion of whom visit many different places in Greece during the summertime.’

The authors state that if the virus was increasing in prevalence in the tourist destinations, this would be detected in the Attica residents. This claim has two major logical flaws: (a) there is significant internal movement of Attica residents from tourist destinations back to Attica during most of the time span of the third period; and (b) their phylogeographic methodology is already valid and proven as reliable for such an induction. Both these claims are unsupported, by statistical evidence as well as common knowledge in Greece. As noted above, Attica residents travel back from their summer vacations well within September, which means that (a) would be valid only if the experimental protocol in the study included sample data from October.

2.7 Additional contradictory information

In this part, some supplementary sources are cited here for examining the inference validity and documented events/facts in relation to the main hypothesis of the study.

On December 1st, 2020, Information Society S.A.⁴, the Greek government agency for ICT infrastructure, awarded a contract⁵ to Price Waterhouse Cooper (PwC) for the data analysis and visual tools regarding the national tracking of the epidemic. In the justification clause (pg.5), the agency clearly

⁴<https://www.ktpae.gr>

⁵Source: <http://vouliwatch.gr>

Ειδικότερα, η επανεκκίνηση της τουριστικής δραστηριότητας σε συνδυασμό με το άνοιγμα των συνόρων έχει αυξήσει σημαντικά τις ροές ξένων πολιτών προς την Ελλάδα, με συνέπεια να έχουν αυξηθεί και τα ενεργά κρούσματα. Οι πεπερασμένες δυνατότητες των τεστ και η ανάγκη για διεξοδική ιχνηλάτηση έχουν ορομολογήσει μια σειρά από ψηφιακές δράσεις από το Υπουργείο Ψηφιακής Διακυβέρνησης προκειμένου να διαμορφωθεί ένα ισχυρό τεχνολογικό υπόβαθρο το οποίο θα στηρίξει την αύξηση της τουριστικής δραστηριότητας.

Figure 11: Quote from the official document of the Information Society S.A. (in Greek).

states that the lifting of the travel restrictions and the opening of the borders in the summer period resulted in the ‘...*increase of active infections.*’ Fig. 11 presents the exact paragraph from the document (in Greek).

This is not directly associated with the paper in review, but it is evidence that even the central Greek authorities recognize that the opening of the borders during the summer increased the number of active infections.

It should also be noted that the numbers presented by the authors regarding the proportion of imported infections during the summer period seems to be much lower than the official daily data reveal. For example, on a single day, September 5th, 2020, of the 187 new confirmed cases 31 (16.58%) were identified at the border checks⁶. By contrast, by this time (March 16th, 2021) the travel restrictions and border checks are much more strict and with more extensive testing than during the summer (2020), with similar number of imported infections identified in almost an order of magnitude higher total number of new daily confirmed cases, namely 27 in 1,533 (1.76%)⁷. This discrepancy is further enhanced by the fact that during the summer the proportion of random tests conducted on travellers arriving to Greece was about 10-15% of their total number according to officials⁸, which means that there was severe under-reporting of infections in that group.

3 Conclusions

In this short review, detailed comments were provided regarding the core thesis and the supporting evidence presented in the study in question. Several problems were identified in the statistical as-

⁶Source: <http://skai.gr>

⁷Source: <http://eody.gov.gr>

⁸<https://www.civilprotection.gr/en/node/6768>

pects of the experimental protocol employed by the authors, specifically:

1. Spatio-temporal incoherence of the sample data w.r.t. the core hypothesis to be tested.
2. Limited volume of data, putting their statistical significance in question.
3. No control cases for comparing the overall evolution profile of the virus spread in Greece during the time periods examined.
4. No justification provided regarding drawbacks of the experimental protocol, including the lack of information about the origin of potential imported cases and the exclusion of samples obtained at border control areas.
5. High statistical correlation between arrivals and infections in Greece during the summer, not commented by the authors.
6. Additional contradictive information from that same time periods which invalidate the authors’ core thesis.

Since the specific publication is not yet submitted for peer review but yet it is of high impact, it is expected that these drawbacks will be addressed successfully by the authors. This commentary provides some hints towards this direction.

References

- [1] E.G. Kostaki, G.A. Pavlopoulos, K.M. Verrou, and et.al. Molecular epidemiology of sars-cov-2 in greece reveals low rates of onward virus transmission after lifting of travel restrictions based on risk assessment during summer 2020. *medRxiv*, 2021.