

## Bioinformatic analysis of the emergence of SARS-CoV-2 in Libya in relation to neighboring countries

Samira Alshafah<sup>1</sup>, Youssef.S.H Fhil Alboum<sup>2</sup>

<sup>1</sup> Computer Department, Faculty of Education, University of Zawia, Libya.

<sup>2</sup> Higher Institute Of Marine Sciences Techniques Sabratha.

Corresponding author email : [samira\\_7\\_8@yahoo.com](mailto:samira_7_8@yahoo.com)

### Abstract

This study aimed to identify the possible transmission pathways of SARS-CoV-2 into Libya and to determine the countries that were most likely to be sources of viral introduction into the country by analyzing the genetic and temporal relationships among viral isolates from Libya and selected neighboring countries. The study adopted a descriptive-analytical approach based on bioinformatics and phylogenetic analysis. SARS-CoV-2 spike protein sequences were retrieved from the NCBI Virus Hub using specific criteria for sequence completeness and quality. A total of 1,426 spike protein sequences were obtained, including 17 sequences from Libya, 86 from Tunisia, 29 from Morocco, 957 from Egypt, and 337 from Italy. Multiple sequence alignment was performed using the MAFFT program through the NGPhylogeny platform, followed by phylogenetic tree reconstruction to investigate the genetic relationships among viral isolates. The collection dates of closely related sequences were also compared to infer possible directions of viral transmission. The results showed that Libyan isolates repeatedly clustered genetically with isolates from Tunisia, Italy, and Morocco. In several clusters, Tunisian isolates had earlier collection dates than closely related Libyan isolates, indicating a likely transmission pathway from Tunisia to Libya. The findings suggest that Tunisia was the most likely major source of SARS-CoV-2 introduction into Libya during the period investigated, with limited evidence supporting transmission from Italy and Morocco to Libya. In contrast, the observed data indicated that viral transmission between Libya and Egypt was predominantly from Libya to Egypt. These findings demonstrate the value of integrating genetic, phylogenetic, and temporal analyses for investigating viral transmission pathways and supporting epidemiological surveillance and preparedness for future infectious disease outbreaks.

**Keywords:** SARS-CoV-2; COVID-19; bioinformatics; genetic analysis; viral transmission; molecular evolution of COV-19

## تحليل معلوماتي حيوي لظهور فيروس كورونا المستجد (SARS-CoV-2) في ليبيا وعلاقته بالدول المجاورة

### الملخص

هدفت هذه الدراسة إلى تحديد المسارات المحتملة لانتقال فيروس SARS-CoV-2 إلى ليبيا، وتحديد الدول الأكثر احتمالاً أن تكون مصدرًا لإدخال الفيروس إلى ليبيا، وذلك من خلال تحليل العلاقات الوراثية والزمنية بين العزلات الفيروسية في ليبيا وعدد من الدول المجاورة. اتبعت الدراسة المنهج الوصفي التحليلي القائم على المعلوماتية الحيوية والتحليل التطوري (Phylogenetic Analysis)، حيث جُمعت تسلسلات بروتين السطح (Spike protein) لفيروس SARS-CoV-2 من قاعدة بيانات NCBI Virus Hub، بعد تطبيق معايير محددة لاختيار التسلسلات الكاملة وتقليل التسلسلات ذات الأحرف الغامضة. شملت مجموعة البيانات 1426 تسلسلاً بروتينياً، منها 17 تسلسلاً من ليبيا، و86 من تونس، و29 من المغرب، و957 من مصر، و337 من إيطاليا. وتمت مقارنة التسلسلات باستخدام المحاذاة المتعددة للتسلسلات (Multiple Sequence Alignment) بواسطة برنامج MAFFT من خلال منصة NGPhylogeny، ثم أُعيد بناء شجرة تطورية لتحليل درجة التقارب الوراثي بين العزلات، مع مقارنة تواريخ جمع العينات لتحديد اتجاهات الانتقال المحتملة. أظهرت النتائج أن العزلات الليبية ارتبطت وراثياً بصورة متكررة بعزلات من تونس وإيطاليا والمغرب، وأن العزلات التونسية ظهرت في عدد من المجموعات التطورية بتاريخ جمع أسبق من العزلات الليبية القريبة منها. وتشير هذه النتائج إلى أن تونس كانت المصدر الأكثر احتمالاً لإدخال SARS-CoV-2 إلى ليبيا خلال الفترة المدروسة، مع وجود أدلة محدودة على انتقال بعض العزلات من إيطاليا والمغرب إلى ليبيا، في حين أشارت البيانات إلى أن اتجاه الانتقال بين ليبيا ومصر كان في الغالب من ليبيا إلى مصر. وتبرز الدراسة أهمية الجمع بين التحليل الجيني والتحليل الزمني في تتبع مصادر وانتقال الفيروسات ودعم جهود المراقبة الوبائية والاستعداد لمواجهة الأوبئة المستقبلية.

## Introduction

The emergence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in late 2019 led to one of the most severe public health crises in modern history [1, 2]. As a single-stranded positive-sense RNA virus, SARS-CoV-2 undergoes continuous genetic evolution driven by mutation and recombination, leading to the rapid global spread of diverse viral lineages through cross-border mobility and international travel [1, 3]. Among the viral structural proteins—including the envelope (E), membrane (M), and nucleocapsid (N) proteins—the spike (S) glycoprotein plays a pivotal role in viral entry by binding to the host angiotensin-converting enzyme 2 (ACE2) receptor [1, 7]. Because the spike protein is the primary target for neutralising antibodies and host immune responses, tracking amino acid substitutions and structural mutations within this region is essential for monitoring viral fitness, immune evasion, and lineage diversification [1, 3, 7].

Molecular epidemiology and bioinformatics have become fundamental tools for reconstructing viral evolutionary trajectories and tracing transmission pathways [2, 3]. By conducting phylogenetic analyses and comparative sequence evaluations on publicly available platforms—such as NCBI Virus Hub and GISAID—researchers can infer cross-border transmission routes, identify super-spreading lineages, and determine primary sources of viral importation [2, 8, 10]. In resource-constrained or logistically challenged settings, integrating viral metadata (such as sample collection dates and geographic origin) with sequence alignment algorithms provides a high-resolution framework to complement traditional epidemiological surveillance [2, 9].

In North Africa, the dynamics of the COVID-19 pandemic were heavily shaped by cross-border movement, trade channels, and regional migration flows [4, 5]. Libya occupies a strategically critical geographic position, connecting North Africa, Sub-Saharan migration corridors, and Southern Europe via the Mediterranean Sea [5, 14]. Following the detection of the first laboratory-confirmed COVID-19 case in Libya in late March 2020, the country experienced multiple epidemic waves influenced by varying degrees of public health measures, border closures, and international travel restrictions [5, 6].

Despite these challenges, early molecular studies provided initial insights into viral lineages circulating in Libya [5, 6]. Fillo et al. [5] demonstrated that early SARS-CoV-2 introductions into Libya originated from multiple geographic regions, including North

Africa, the Middle East, and Asia. Subsequent genomic surveillance by Alhudiri et al. [6] identified the widespread circulation of the B.1.525 (Eta) lineage in early 2021, underscoring the dynamic turnover of circulating variants within the Libyan population. Furthermore, studies on migrant populations traveling through the Libyan route confirmed the active circulation and transboundary movement of distinct viral clades across the Mediterranean basin [14].

However, despite these foundational genomic investigations, there remains a critical gap in quantifying the exact directional transmission pathways between Libya and its immediate neighboring countries—namely Tunisia, Egypt, Morocco, and geographically adjacent Southern European nations such as Italy [4, 5]. Understanding whether viral lineages were primarily imported into Libya or exported to surrounding countries is crucial for regional health security and future pandemic preparedness [3, 4].

To address this gap, the present study applies bioinformatics and phylogenetic approaches to analyze 1,426 SARS-CoV-2 spike protein sequences retrieved from NCBI Virus, encompassing Libya, Tunisia, Morocco, Egypt, and Italy [8, 9]. By coupling sequence alignment and phylogenetic tree reconstruction with a systematic comparison of precise temporal collection dates, this study aims to evaluate the genetic relationships among regional isolates, reconstruct potential cross-border transmission routes, and pinpoint the primary geographic sources of SARS-CoV-2 introductions into Libya [1, 8].

#### **Related Work (Previous Studies)**

Several studies have employed genomic sequencing and phylogenetic analysis to investigate the evolution and transmission dynamics of SARS-CoV-2 worldwide. Early genomic investigations demonstrated the usefulness of viral genome sequencing in identifying transmission chains and tracing the international spread of the virus.

Lu et al. [1] analyzed the genomic characteristics of SARS-CoV-2 and revealed its close genetic relationship with bat coronaviruses. Their findings provided important evidence regarding the origin and evolutionary history of the virus and emphasized the significance of genomic surveillance in monitoring emerging infectious diseases.

Hadfield et al. [2] developed the Nextstrain platform, an open-source tool for real-time tracking of pathogen evolution. The platform enables continuous phylogenetic analysis

and visualization of viral transmission patterns across different countries and regions, making it a valuable resource for global genomic surveillance.

Deng et al. [3] applied genomic epidemiology approaches to investigate SARS-CoV-2 transmission in Northern California. Their phylogenetic analysis revealed multiple independent introductions of the virus into the region rather than the dominance of a single transmission lineage.

In North Africa, genomic surveillance studies have explored the diversity and circulation of SARS-CoV-2 lineages. Khlif et al. [4] analyzed Tunisian SARS-CoV-2 genomes and reported the presence of multiple viral lineages introduced through international travel and regional transmission networks, highlighting the role of cross-border movement in viral dissemination.

Fillo et al. [5] conducted one of the earliest genomic characterizations of SARS-CoV-2 isolates collected in Libya. Their phylogenetic analysis suggested that the virus was introduced into Libya from multiple geographic regions, including North Africa, the Middle East, and Asia, emphasizing the impact of international mobility on the early spread of COVID-19 within the country.

Alhudiri et al. [6] investigated SARS-CoV-2 variants circulating in Libya through whole-genome sequencing. Their study identified the widespread circulation of the B.1.525 lineage during early 2021 and demonstrated the importance of genomic surveillance in detecting emerging variants and monitoring their transmission within the Libyan population.

Together, these studies demonstrate that genomic and phylogenetic approaches are essential for reconstructing transmission pathways, identifying viral introductions, and understanding the evolutionary dynamics of SARS-CoV-2. The present study extends this work by specifically examining the phylogenetic relationships between Libyan isolates and neighboring countries using spike protein sequence analysis and temporal comparison of collection dates.

Despite these efforts, limited studies have specifically examined the phylogenetic relationships between Libyan SARS-CoV-2 isolates and those from neighboring countries. Therefore, the present study contributes to the existing literature by combining spike protein sequence comparison, phylogenetic tree reconstruction, and temporal

analysis to infer possible transmission routes into Libya and evaluate the role of neighboring countries in the introduction and spread of SARS-CoV-2.

### Material and method

The research was done on the basis of annotated SARS-CoV-2 corona viruses [7] found in the NCBI virus hub [8]. The database was queried by selecting *SARS-CoV-2 coronavirus*, which yielded more than 9 million nucleotides. Sequence filtering was applied using the following criteria: nucleotide completeness was restricted to complete isolates, and the maximum number of ambiguous characters was limited to one. Geographic filtering was performed by selecting the African region and including isolates from Libya, Tunisia, Morocco and Egypt, as well as Italy as the nearest European country. It is assumed that the largest number of travelers and contacts with Libyan citizens came from these countries. Under genome organization filters, only sequences annotated as containing surface glycoproteins were selected. Together with protein codes, collection date and release date were download as additional metadata.

A total of 1426 spike protein sequences were obtained, including 17 from Libya, 86 from Tunisia, 29 from Morocco, 957 from Egypt and 337 from Italy.

The idea how to control and determine the source of the virus was to find set of viruses with similar sequences in the observed countries and compare their collection date.

Multiple sequence alignment was used to determine the similarity of virus sequences.

Multiple sequence alignment was performed on this dataset using the MAFFT program available through the NGPhylogeny platform [9]. NGPhylogeny provides integrated phylogenetic tree construction based on aligned protein sequences. This approach allows the identification of phylogenetic relationships between isolates from different countries and enables the detection of proteins from other regions that are closely related to Libyan isolates.

### Results and Discussion

A total of 1,426 SARS-CoV-2 spike protein sequences were obtained from the NCBI Virus Hub after applying the specified sequence quality and completeness criteria. The dataset included 17 sequences from Libya, 86 from Tunisia, 29 from Morocco, 957 from Egypt, and 337 from Italy. Multiple sequence alignment was performed using MAFFT through the NG Phylogeny platform, followed by phylogenetic tree reconstruction to

investigate the genetic relationships among the viral isolates. Collection dates were subsequently compared among closely related sequences to infer possible transmission directions.

The phylogenetic analysis demonstrated that the Libyan SARS-CoV-2 spike protein sequences were distributed within several clusters containing sequences from Tunisia, Italy, Morocco, and Egypt. The repeated clustering of Libyan sequences with isolates from different countries indicates that the introduction of SARS-CoV-2 into Libya was unlikely to have originated from a single transmission event. Instead, the observed phylogenetic pattern suggests multiple introductions from different geographical sources.

Collection dates from proteins annotated as Libyan source with their close neighbours based on the phylogenetic tree (Figure 1) and ITOL tool (Figure 2) are shown in Table 1. Collection date of each Libyan protein is compared with collection date of all close neighbour proteins. If the isolate to which that protein belongs was collected in neighboring countries before it was collected in Libya, then it can be said that that virus isolate was transferred to Libya from a neighboring country and vice versa.

Based on the collection dates annotated in the phylogenetic tree, the direction of SARS-CoV-2 transmission among Morocco, Tunisia, Egypt, Italy and Libya was inferred using multiple spike protein sequences rather than relying on a single representative example.

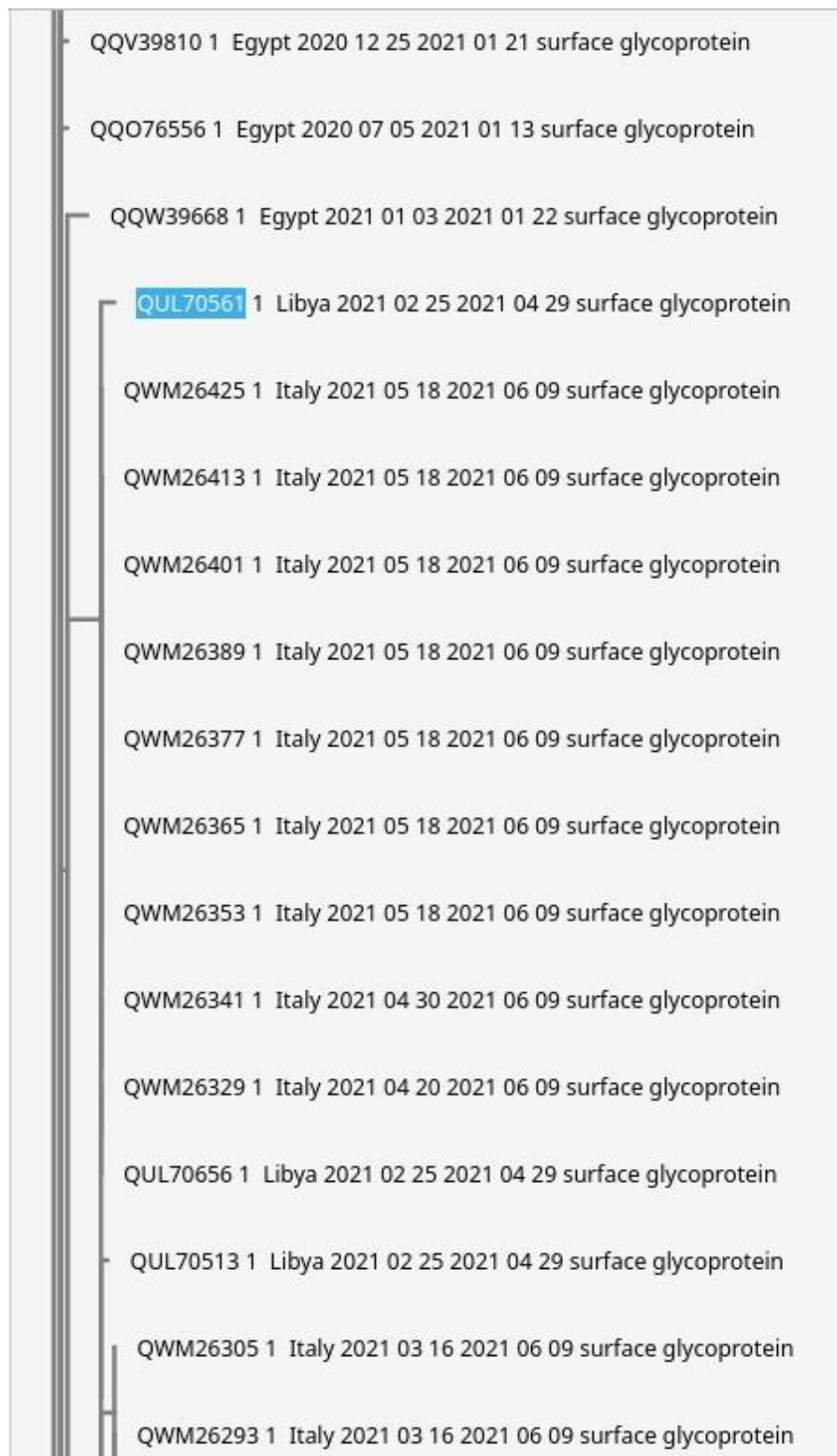


**Figure 1.** Phylogenetic tree (part) with proteins in Libya and their close neighbors. First date after country name is virus collection date.

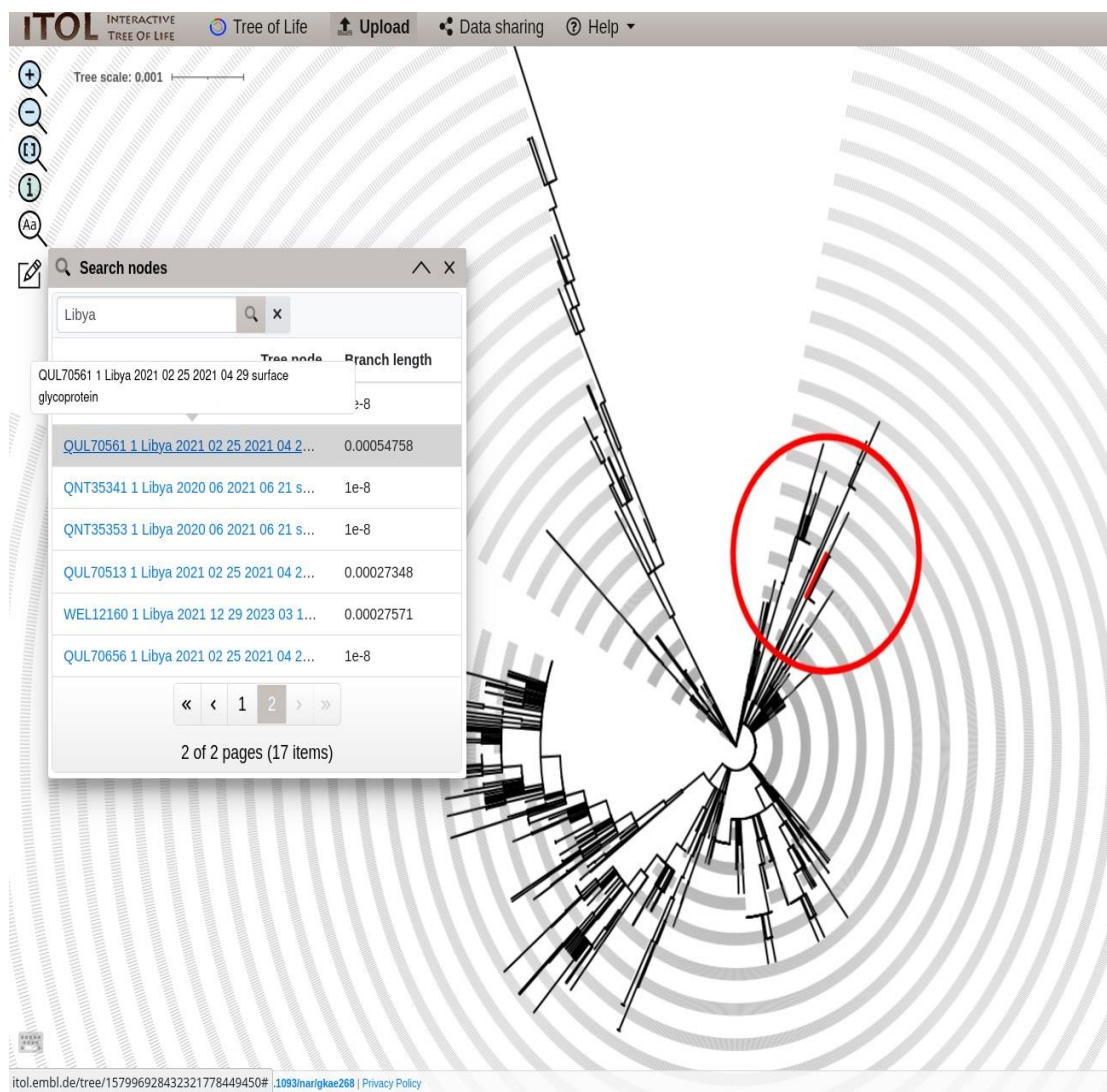


**Figure 2.** Phylogenetic tree displayed using ITOL tool. Red Circle marks the part of the tree where Libyan protein QUL70561.1 is located. The exact position of the protein in the tree is indicated by the red line in the middle of the circle.

**Table 1.** Virus transfer among countries. Red - source country; Green - destination country.

| Libyan proteins |                 | Neighbor country |                 |
|-----------------|-----------------|------------------|-----------------|
| Protein id      | Collection date | Country          | Collection date |
| QNT35353.1      | 2020-06         | Tunisia          | 2020_03_24      |
| QNT35341.1      | 2020-06         | Tunisia          | 2020_03_24      |
| QNT35329.1      | 2020-06         | Tunisia          | 2020_03_24      |
| QNT35317.1      | 2020-06         | Tunisia          | 2020_03_24      |
| QNT35305.1      | 2020-06         | Tunisia          | 2020_03_24      |
| QNT35401.1      | 2020-06         | Tunisia          | 2020_03_24      |
| WEL11988.1      | 2022-02-16      | Italy            | 2021-12-20      |
| QNT35413.1      | 2020-06         | Tunisia          | 2020_03_24      |
| QNT35389.1      | 2020-06         | Tunisia          | 2020_07_07      |
|                 |                 | Italy            | 2020-06-29      |
| WEL12160.1      | 2021-12-29      | Italy            | 2021-12-20      |
|                 |                 |                  | 2021-12-06      |
|                 |                 |                  | 2021-08-24      |
| QNT35377.1      | 2020-06         | Tunisia          | 2020_03_24      |
| QUL70561.1      | 2021-02-25      | Italy            | 2021-05-18      |
|                 |                 |                  | 2021-04-30      |
| QUL70561.1      | 2021-02-25      | Italy            | 2021-05-18      |
|                 |                 | Egypt            | 2021-03-01      |
| QUL70656.1      | 2021-02-25      | Italy            | 2021-04-20      |
| QUL70703.1      | 2021-02-25      | Egypt            | 2020-05-01      |
|                 |                 | Morocco          | 2020-12-25      |
|                 |                 |                  | 2021-01-09      |
|                 |                 |                  | 2020-07-01      |
|                 |                 | Italy            | 2020-02-21      |
| QUL70822.1      | 2021-02-25      | Egypt            | 2021-05-17      |
|                 |                 |                  | 2021-05-14      |
|                 |                 | Italy            | 2021-06-12      |
| QUL70513.1      | 2021-02-25      | Italy            | 2021-03-16      |
|                 |                 |                  | 2021-05-18      |
| QUL70525.1      | 2021-02-25      | Egypt            | 2021-05-01      |
|                 |                 | Italy            | 2021-03-10      |
| QUL70525.1      | 2021-02-25      | Italy            | 2020-12-19      |

The phylogenetic structure consistently shows that Libyan sequences cluster with Italian and Tunisian isolates across several branches of the tree, while temporal information provides key evidence for identifying the most likely source of viral introduction. Across multiple mixed clusters, Tunisian sequences frequently exhibit earlier collection dates compared to their closely related Libyan counterparts. In these clusters, Libyan spike

protein sequences are positioned downstream of Tunisian isolates with very short branch lengths, indicating recent divergence from a common ancestor. This repeated temporal precedence of Tunisian isolates strongly supports the hypothesis that Tunisia acted as a primary source of SARS-CoV-2 introduction into Libya for several viral lineages. There are no evidences (according to observed collection dates) that some is virus transmitted in the opposite direction.

Additionally, there are cluster of Moroccan sequences that are cluster near Libyan isolate QUL70703.1 and some viruses from these cluster are also transmitted to Libya in the end of 2020 and in the first half of 2021 year. This data indicates that the introduction of the virus into Libya during 2020 was mainly from these countries, consistent with regional transmission dynamics.

It is interesting relationships with Italia and Egypt as virus source countries. While only in period dated around may 2020 year some viruses are transferred to Libya from Egypt, in all other period viruses from Libya are "travelled" to Egypt, despite the significantly higher number of viruses collected in Egypt (957) than in Libya (17). On the other hand, with Italia there are some kind of "virus interchange" - some viruses are transferred from Italy to Libya while some other are transmitted in opposite direction. Very interesting is protein QUL70525.1 who has similar proteins (compared to sequence) in Italia before and after date when it was collected in Libya. This suggests that virus with protein QUL70525.1 was exported to Italy where it mutated and was then re-imported the Libya. Comparative analysis of phylogenetic clustering, together with available collection dates embedded in the FASTA headers, enabled inference of possible transmission routes. Notably, the Tunisian isolate had an earlier collection date than both the Libyan, Moroccan and Egyptian isolates, suggesting a likely transmission pathway from Tunisia and other countries.

### Comparison with Previous Studies

The finding that SARS-CoV-2 was introduced into Libya through multiple international transmission pathways is consistent with previous genomic investigations. Fillo et al. [5], who characterized SARS-CoV-2 genomes collected in Libya, reported that the virus was introduced into Libya from multiple geographical regions, including North Africa, the Middle East, and Asia. The present study supports the concept of multiple introductions

but provides additional evidence suggesting that Tunisia played a particularly important role among the countries examined in the present analysis.

The current findings are also consistent with genomic surveillance conducted in Tunisia. Ben Sassi et al. [11] analyzed SARS-CoV-2 genomes collected in Tunisia between March 2020 and March 2021 and identified multiple lineage introductions during the early phase of the pandemic. Their study also reported substantial lineage diversity, particularly in northern Tunisia. ([ScienceDirect](#)) This observation is compatible with the present study because the presence of multiple viral lineages in Tunisia provides a plausible genetic background for the repeated clustering of Tunisian and Libyan isolates.

The present findings are further supported by Deng et al. [12], who demonstrated through genomic and phylogenetic analysis that SARS-CoV-2 was introduced into Northern California through multiple independent transmission events rather than through a single predominant lineage. Their analysis identified at least seven distinct introductions. ([PubMed Central \(PMC\)](#)) Although the geographical setting is different, both studies demonstrate the value of phylogenetic analysis in identifying multiple introductions that may not be apparent from conventional epidemiological data alone.

The results of the present study also complement the findings of Alhudiri et al. [13], who used whole-genome sequencing to investigate SARS-CoV-2 circulation in Libya and reported widespread circulation of the B.1.525 lineage in February 2021. Their findings demonstrate the importance of genomic surveillance in identifying circulating viral lineages within Libya. ([tandfonline.com](#)) The present study extends this perspective by focusing specifically on the phylogenetic relationships between Libyan spike protein sequences and sequences from neighboring and geographically connected countries.

## Conclusion

This study highlights the importance of integrating genomic and phylogenetic analysis with temporal collection dates to trace viral transmission routes and identify cross-border origins of infection. Based on the bioinformatic analysis of SARS-CoV-2 spike protein sequences from Libya and neighboring countries (Tunisia, Morocco, Egypt, and Italy), the following key conclusions were drawn:

1. Multiple Viral Introductions: The distribution of Libyan SARS-CoV-2 spike protein sequences across several distinct phylogenetic clusters demonstrates that the virus was introduced into Libya through multiple independent transmission events from diverse geographic sources rather than a single introduction.
2. Tunisia as a Primary Source: Temporal and phylogenetic comparisons identified Tunisia as the predominant primary source of SARS-CoV-2 introduction into Libya during the period investigated. Tunisian isolates consistently appeared in basal positions within phylogenetic clusters with earlier collection dates relative to closely related downstream Libyan isolates.
3. Transmission Dynamics with Other Neighboring Countries:
  - Italy and Morocco: Limited evidence supports viral transmission from Morocco and Italy to Libya, along with observed bidirectional interchange with Italy (including potential export, mutation, and re-importation of certain viral lineages).
  - Egypt: Despite the significantly larger sequence dataset available from Egypt, transmission between Libya and Egypt was predominantly directed from Libya to Egypt, with limited transmission from Egypt to Libya restricted to early 2020.
4. Utility of Spike Protein Analysis: The bioinformatic workflow utilizing spike protein sequences, multiple sequence alignment via MAFFT, and phylogenetic tree reconstruction using NG Phylogeny and ITOL proved to be an effective approach for molecular epidemiological surveillance and reconstructing transmission chains.

### Recommendations

Based on the findings regarding the regional transmission dynamics of SARS-CoV-2, the following recommendations are proposed:

1. Strengthen Cross-Border Genomic Surveillance: Prioritize and enhance molecular screening and genomic surveillance at key land and air entry points, particularly along high-traffic borders such as the Libya–Tunisia border, to detect and mitigate the early introduction of novel imported variants.
2. Establish Regional Data-Sharing Networks: Build collaborative regional networks among North African and Southern European public health authorities to facilitate real-time sharing of viral genomic sequences and associated metadata (e.g., collection dates and geographic origin) to support proactive outbreak response.

3. Expand Local Genomic Sequencing Capacity: Invest in domestic sequencing infrastructure and capacity-building within Libyan research centers and public health laboratories to increase the number of complete SARS-CoV-2 sequences submitted to global repositories (e.g., NCBI Virus, GISAID), thereby reducing sampling bias in future evolutionary analyses.
4. Utilize Whole-Genome Sequencing (WGS): Conduct future research using whole-genome sequencing datasets to complement spike protein analysis, providing higher resolution for lineage classification and mutational profiling.
5. Integrate Genomic Data with Epidemiological and Mobility Metadata: Combine phylogenetic findings with patient travel histories, demographic data, and human mobility patterns to better understand domestic transmission chains and community spread within Libyan municipalities.

## References:

- [1] Roujian Lu, Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., ... & Tan, W. (2020). *Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding*. The Lancet, 395(10224), 565–574.
- [2] James Hadfield, Megill, C., Bell, S. M., Huddleston, J., Potter, B., Callender, C., Sagulenko, P., Bedford, T., & Neher, R. A. (2018). *Nextstrain: Real-time tracking of pathogen evolution*. Bioinformatics, 34(23), 4121–4123.
- [3] Xianding Deng, Gu, W., Federman, S., du Plessis, L., Pybus, O. G., Faria, N. R., Wang, C., ... & Chiu, C. Y. (2020). *A Genomic Survey of SARS-CoV-2 Reveals Multiple Introductions into Northern California without a Predominant Lineage*. Science, 369(6503), 582–587.
- [4] Khelif, K., et al. (2022). *Phylogenetic and amino acid signature analysis of the SARS-CoV-2 lineages circulating in Tunisia*. Virus Genes, 58, 266–278.
- [5] Silvia Fillo, Giordani, F., Monte, A., Faggioni, G., De Santis, R., D'Amore, N., Palomba, S., Hamdani, T., Taloa, K., & Jumaa, A. B. (2021). *Genomic Characterization and Phylogenetic Analysis of SARS-CoV-2 in Libya*. Microbiology Research, 12(1), 138–149.
- [6] Inas M. Alhudiri, Ramadan, A. M., Ibrahim, K. M., Abdalla, A., Eljilani, M., Salem, M. A., Elgheriani, H. M., El Meshri, S. E., & Elzagheid, A. (2021). *Whole-genome*

- sequencing of SARS-CoV-2 showed wide spread of B.1.525 in February 2021 Coronavirus disease in Libya. *Libyan Journal of Medicine*, 16(1), 2001210.
- [7] World Health Organization (WHO). (2020). Coronavirus disease (COVID-19) technical guidance: Surveillance and case definitions. Available online: <https://www.who.int/health-topics/coronavirus>
- [8] NCBI Virus hub: <https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/>, accessed on 04.05.2026
- [9] Frédéric Lemoine, Damien Correia, Vincent Lefort, Olivia Doppelt-Azeroual, Fabien Mareuil, Sarah Cohen-Boulakia, Olivier Gascuel, NGPhylogeny: new generation phylogenetic services for non-specialists, *Nucleic Acids Research*, Volume 47, Issue W1, 02 July 2019, Pages W260–W265, <https://doi.org/10.1093/nar/gkz303>
- [10] Letunic, I., & Bork, P. (2021). Interactive Tree Of Life (ITOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, 49(W1), W293–W296.
- [11] Ben Sassi, M., Ferjani, S., Mkada, I., et al. (2022). Phylogenetic and amino acid signature analysis of the SARS-CoV-2s lineages circulating in Tunisia. *Infection, Genetics and Evolution*, 102, 105300. DOI: 10.1016/j.meegid.2022.105300.
- [12] Deng, X., Gu, W., Federman, S., et al. (2020). Genomic surveillance reveals multiple introductions of SARS-CoV-2 into Northern California. *Science*, 369(6503), 582–587. DOI: 10.1126/science.abb9263.
- [13] Alhudiri, I. M., Ramadan, A. M., Ibrahim, K. M., et al. (2021). Whole-genome sequencing of SARS-CoV-2 showed wide spread of B.1.525 in February 2021 in Libya. *Libyan Journal of Medicine*, 16(1), 2001210.
- [14] Tramuto, F., Reale, S., Lo Presti, A., Vitale, F., Pulvirenti, C., Rezza, G., ... & Stefanelli, P. (2021). Genomic analysis and lineage identification of SARS-CoV-2 strains in migrants accessing Europe through the Libyan route. *Frontiers in Public Health*, 9, 632645.