

# Web Tools for Genomic Epidemiology - Paraguay 2023

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## Background

Genomic epidemiology aims to understand the emergence and dissemination of high-risk clones within pathogen populations with the ultimate goal of implementing evidence-based interventions to protect public health. High-risk clones are subpopulations/strains/variants of a pathogen that carry risk elements, such as antibiotic resistance or virulence determinants, and thus pose a potential risk to public health.

The increased genetic resolution afforded by genomic data is useful from global to local geographic scales, and it has proven particularly useful for the investigation of pathogens that exhibit little genetic variation (e.g. Wong *et al.* 2016), and for outbreak investigations (e.g. Hendriksen *et al.* 2011, Eppinger *et al.* 2014). During outbreak investigations (and other genomic epidemiology studies), epidemiological data from patients is collected by healthcare professionals. Antimicrobial susceptibility data, species identification, and any further phenotypic or molecular characterization of the isolates is often generated by the laboratories linked to healthcare facilities and/or by the reference laboratory. Ideally, these different sources of data are stored in a centralised surveillance system and database, such as WHONET. However, these systems rarely incorporate genomic data produced by bioinformaticians. Genomic data may include sequence/assembly quality, genotyping/genosero-typing information, presence/absence of known risk elements, and clustering of the isolates based on genetic similarity –usually in the form of a phylogenetic tree or a minimum spanning-tree.

The job of a genomic epidemiologist often starts by combining data from diverse sources that might not be complete or standardised to facilitate the identification of relevant patterns for meaningful interpretation. Once integrated, the epi, lab, and genomic data can be interrogated over the structure of the tree to identify clusters of interest that will place the isolates in or out of the outbreak.

The **Centre for Genomic Pathogen Surveillance** develops free web applications for data collection, integration, visualisation, and analysis of genomic epidemiology data. **Epicollect5** is a mobile & web application for free and easy data collection. It provides both the web and mobile applications for the generation of forms (questionnaires) and freely hosted project websites for data collection. Projects are created by using the web application at [five.epicollect.net](http://five.epicollect.net), and then downloaded to the device to perform the data collection. Data are collected in the field using multiple devices and all data can be viewed on a central server (via map, tables, and charts). **Data-flo** is a system for customised integration and manipulation of diverse data via a simple drag and drop interface. Data-flo provides a visual method to design a reusable pipeline to integrate, clean, and manipulate data in a multitude of ways, eliminating the need for continuous manual intervention (e.g., coding, formatting, spreadsheet formulas, manual copy-pasting). **Microreact** allows you to upload, visualise and explore any combination of clustering (trees), geographic (map) and temporal (timeline) data. Other metadata variables are displayed in a table. You can specify colours and/or shapes to display on the map, tree and/or timeline. A permanent URL is produced for you to share your Microreact.

## Introduction

Aim: To use web and mobile tools for the collection of epi data in the field, its integration with lab and bioinformatics data, and to finally visualise it together to detect a putative outbreak.

*Note: This exercise is a work of fiction. Any resemblance to a real cholera outbreak is purely coincidental.*

During January and February 2023, fifty-eight patients reported to eight hospitals in Asunción with acute watery diarrhoea and vomiting. *Vibrio cholerae* was isolated from the stool samples of fourty patients (all belong to serotype Inaba), and one colony from each confirmed case was sequenced on Illumina MiSeq. An epidemiological investigation revealed that more than half of the culture-confirmed cases regularly visited the Ñu Guazú Park, where water fountains and other water sources are accessible, and which is crossed by the Arroyo Itay. This suggested that the outbreak could be caused by contaminated water in the park. The epidemiological and demographic data is summarised on the file `epi_data.csv`.

A team of disease detectives will be deployed around Ñu Guazú Park today to investigate the potential source of the outbreak using Epicollect5, data-flo,

and Microreact, free mobile and web applications developed by the Centre for Genomic Pathogen Surveillance for easy data collection, integration and visualisation.

## Learning Outcomes

At the end of this session the participants will be able to:

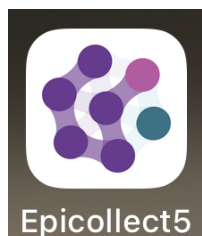
1. Collect data in the field with Epicollect5.
2. Merge epidemiological, laboratory and genomic data with data-flo
3. Create a Microreact project to visualise epidemiological, laboratory and genomic data

### 1. Collect field data with Epicollect

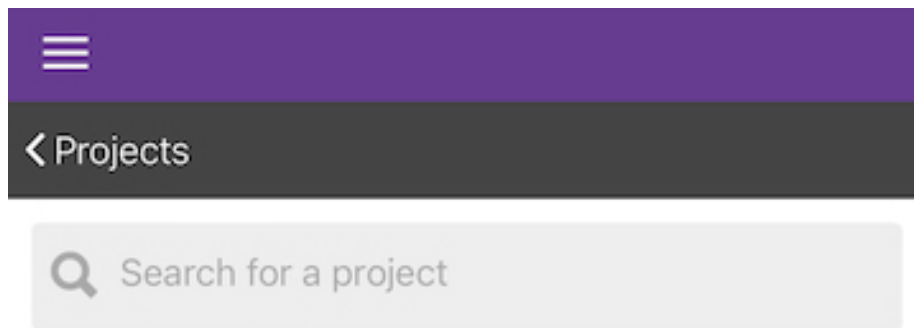
<https://five.epicollect.net/>

The disease detectives will work in pairs, and will be provided with a **collection tube** with a **barcode** sample identifier to collect a sample of water, and will use their **mobile phone** to collect associated metadata in the field.

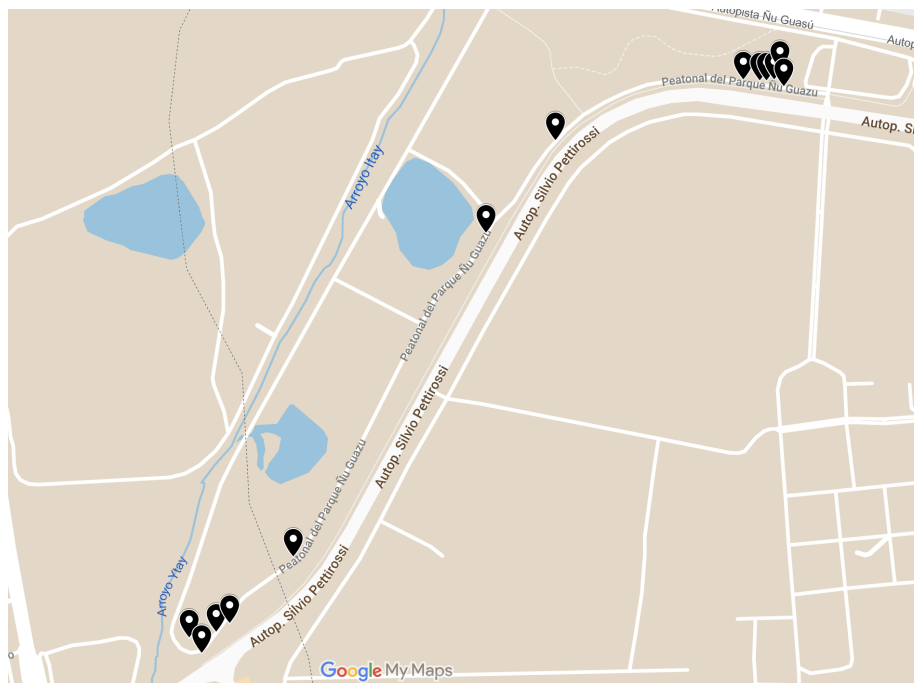
Make sure your phone is connected to WiFi. Open the **Epicollect5** app on your mobile phone.



Add the project **Asuncion 2023 Cholera Outbreak** by typing into the search box –you will need to be connected to WiFi.

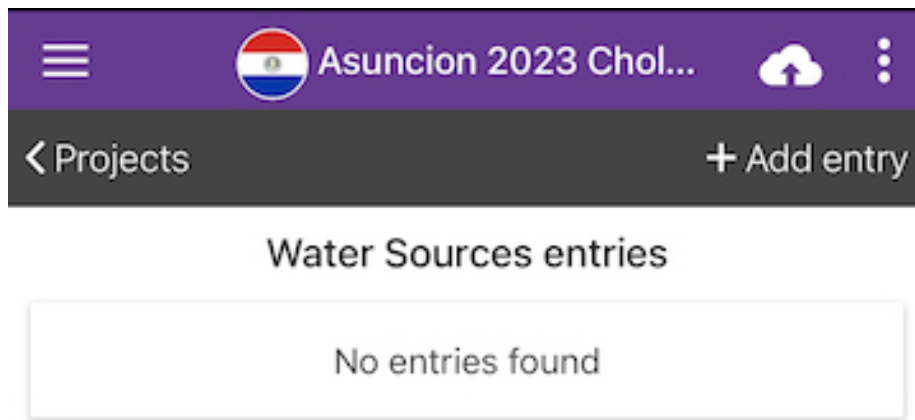


Using the map of the Ñu Guasú park provided, walk to the location of your collection point (water source). Locations are indicated by the red dots labelled a-j. You will not need internet access to collect data in the field.



Open the **Asuncion 2023 Cholera Outbreak** project and add an entry. Follow the form to collect a water sample and associated metadata.

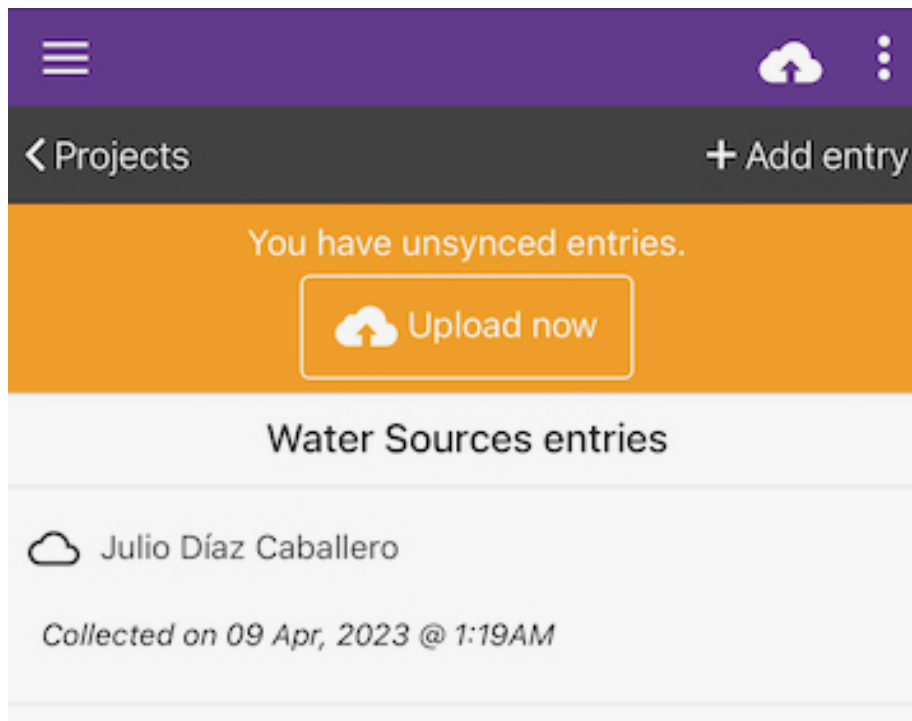




Your team will collect only one water sample, but both of you can collect the associated data on Epicollect5. However, *make sure that only one of you uploads the data later on to avoid duplications.*

Return to the Epidemic Intelligence Center (i.e. the classroom).

One of the pair members only. **Upload your entry (and image)** to the Epicollect5 server –make sure you are connected to the WiFi.



Submit your water sample to the instructor, which will be sent to the reference lab for culture confirmation.

Once all entries are uploaded by the disease detectives we will take a look at the data together on <https://five.epicollect.net/project/asuncion-2023-cholera-outbreak>

Answer the following questions:

1. What is the most common type of water source?
2. Was it possible to collect a sample from all 13 sources? If not, what were the reasons?

## 2. Merge data with Data-flo

<https://data-flo.io/>

*Note: you need to sign-up for data-flo and Microreact. See instructions in the Resources section. Creating your own account will allow you to manage and edit your projects.*

The reference lab has sent you the culture results on an Excel file called **lab\_results.xlsx**. The lab reported that **2 out of 13** water samples from Ñu Guasú park were positive for *Vibrio cholerae*. **This immediately prompted the closure of the 2 water sources.**

Does this confirm that the source of the outbreak was contaminated water from Ñu Guasú park?

One colony from each source was sequenced on Illumina MiSeq by the reference lab. A maximum likelihood phylogenetic tree (**tree.nwk**) was inferred from the genomes of the 34 clinical samples and 2 environmental (water) samples. Six genomes from a previous outbreak (Jan-Feb 2020) were also included in the tree inference and their associated data added to the **epi\_data.csv** file.


The disease detectives now have the information needed for the investigation in the following formats:




- **epi\_data.csv** Epi data from 34 clinical cases and 6 cases from previous outbreak
- **Epicollect project** Metadata of 13 water sources from Ñu Guasú Park
- **lab\_results.xlsx** Culture and serotyping results
- **tree.nwk** Phylogenetic tree of 34 clinical cases, 2 culture-positive water samples, and 6 cases from previous outbreak

The files are located in the VM folder **FOLDER NAME**.




We will combine data from these different sources with a data-flo workflow that takes the files above and the data from the Epicollect project as input, and creates as an output a Microreact project where the data can be visualised.

Open the data-flo workflow (<https://data-flo.io/run?prnvpvmKzq8rDKbPD9ZXAs>)

 TRANSFORMATIONS DOCUMENTATION SIGN OUT

← Asuncion 2023 Cholera Outbreak   

Created: 03/03/2023, 10:27:36 Updated: 09/04/2023, 01:39:59

 INFO  RUN  API

### Description

This is an exercise designed for the WGCAC Genomics and Epidemiological Surveillance of Bacterial Pathogens (Asuncion, Paraguay) 16 - 21 April 2023

### Inputs

epicollect project url (**text**)

lab results excel (**file**)


epi\_data csv (**file**)




tree (**file**)

### Outputs




url (**url**)

Copy the workflow to your own dataflo account.



← Asuncion 2023 Cholera Outbreak   

Created: 03/03/2023, 07:27:36 Updated: 17/04/2023, 09:44:23

 INFO  RUN  API

### Description

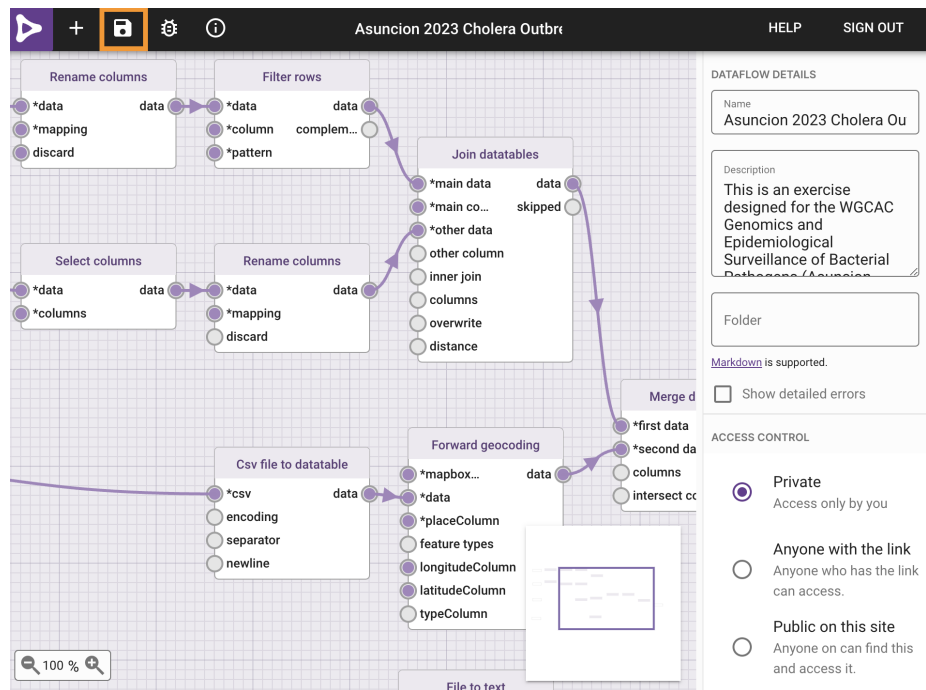
This is an exercise designed for the WGCAC Genomics and Epidemiological Surveillance of Bacterial Pathogens (Asuncion, Paraguay) 16 - 21 April 2023

This will open a copy of this workflow in your dataflo account.

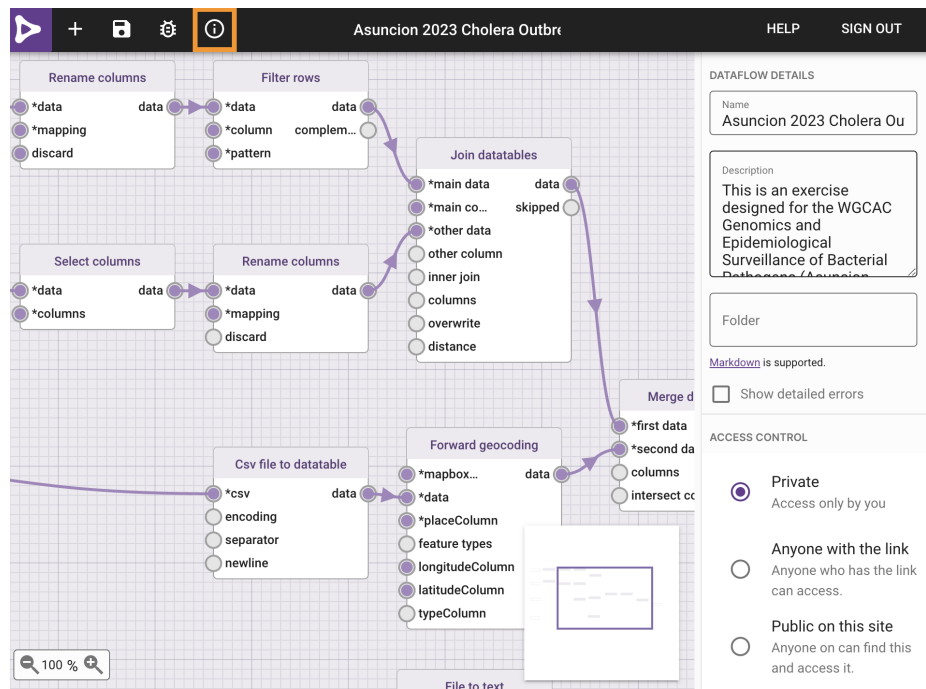


The screenshot shows the Dataflow Builder interface for a project titled "Asuncion 2023 Cholera Outbre". The main workspace displays a "Create microreact project" transformation. A dropdown menu is open, showing a list of inputs: "name", "descripti...", "\*data", "tree", "network", "api", "\*access token" (highlighted), "id column", "timeline column", "latitude column", and "longitude column". The "url" output of the transformation is connected to a "url" input field. On the right, the "ARGUMENT: access token" section shows the "Data type" as "text" and provides a link to the Microreact API documentation. The "BINDING TYPE" section shows "Bind to a value" selected. The "VALUE" section displays the access token: "eyJhbGciOiJIaXLCJlbnMiOiJBMjU2R0NNIn0..FLtr". A "REMOVE BINDING" button is visible below the value field. The top navigation bar includes icons for play, add, save, and help, along with the project name and "HELP" and "SIGN OUT" links. The bottom left corner shows a zoom level of "125 %".

Save your newly edited dataflow project by clicking on the **save** icon.



Now lets go to the implementation page by clicking on the i icon.

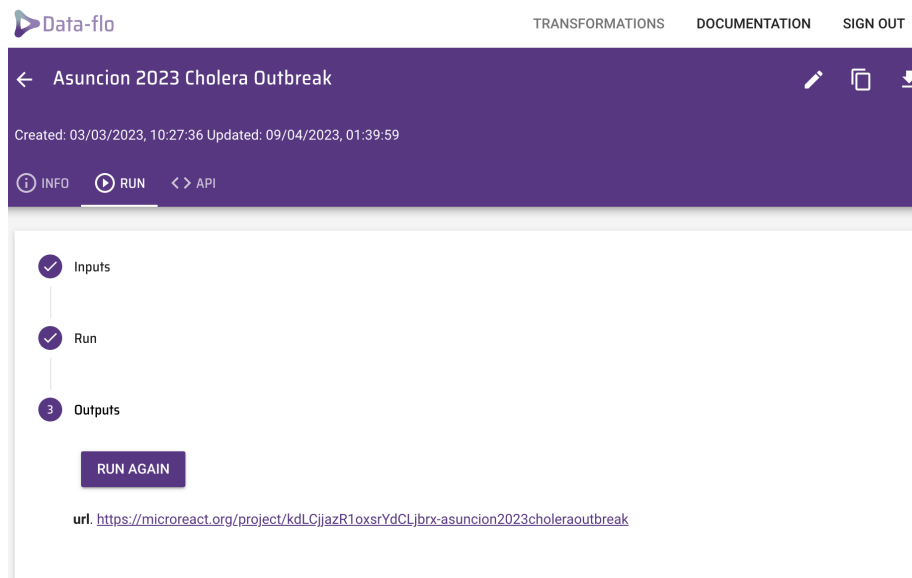


To run the workflow paste the url of the Epicollect5 project (<https://five.epicollect.net/project/asuncion-2023-cholera-outbreak>) and upload the files.

The screenshot shows the Data-flo web interface for a workflow titled "Asuncion 2023 Cholera Outbreak". The interface has a purple header with the Data-flo logo and navigation links for "TRANSFORMATIONS", "DOCUMENTATION", and "SIGN OUT". Below the header, the workflow title is displayed with edit, copy, and download icons. The creation and update timestamps are shown: "Created: 03/03/2023, 10:27:36 Updated: 09/04/2023, 01:39:59". A navigation bar includes "INFO", "RUN" (highlighted with a play icon), and "< > API". The main workspace is divided into three steps: 1. Inputs, 2. Run, and 3. Outputs. In the "Inputs" step, four input fields are visible: "epicollect project url" with the value "https://five.epicollect.net/project/asuncion-2023-cholera-outbreak", "lab results excel" with a "Choose file" button and "lab\_results.xlsx", "epi\_data csv" with a "Choose file" button and "epi\_data.csv", and "tree" with a "Choose file" button and "tree.nwk". A purple "RUN" button is highlighted with an orange rectangle. The "Run" and "Outputs" steps are currently empty.

Click on **Run**. The Outputs box now shows the url of a Microreact project created by data-flo.





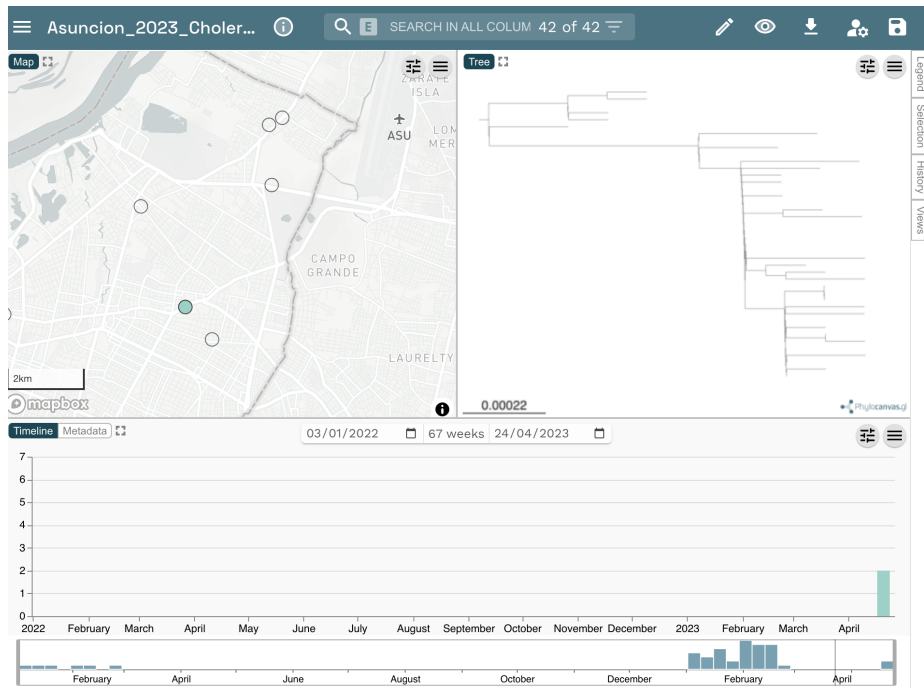
Before you open the Microreact project answer the following questions:

1. If later on we wanted to add additional entries (i.e. water samples) to the Epicollect project, could we use the same data-flo? If so, how would this be beneficial?
2. Do you use/know of any other methods to join data? If so, how do they compare with data-flo?

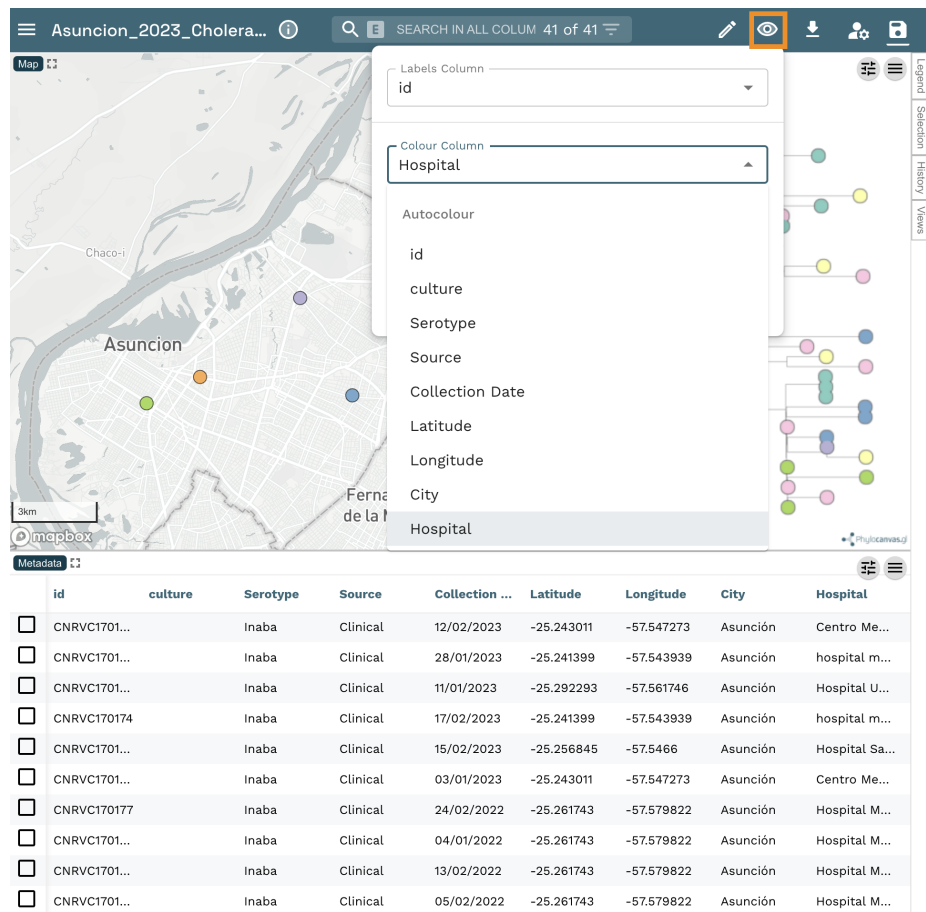
### 3. Microreact

<https://microreact.org/>

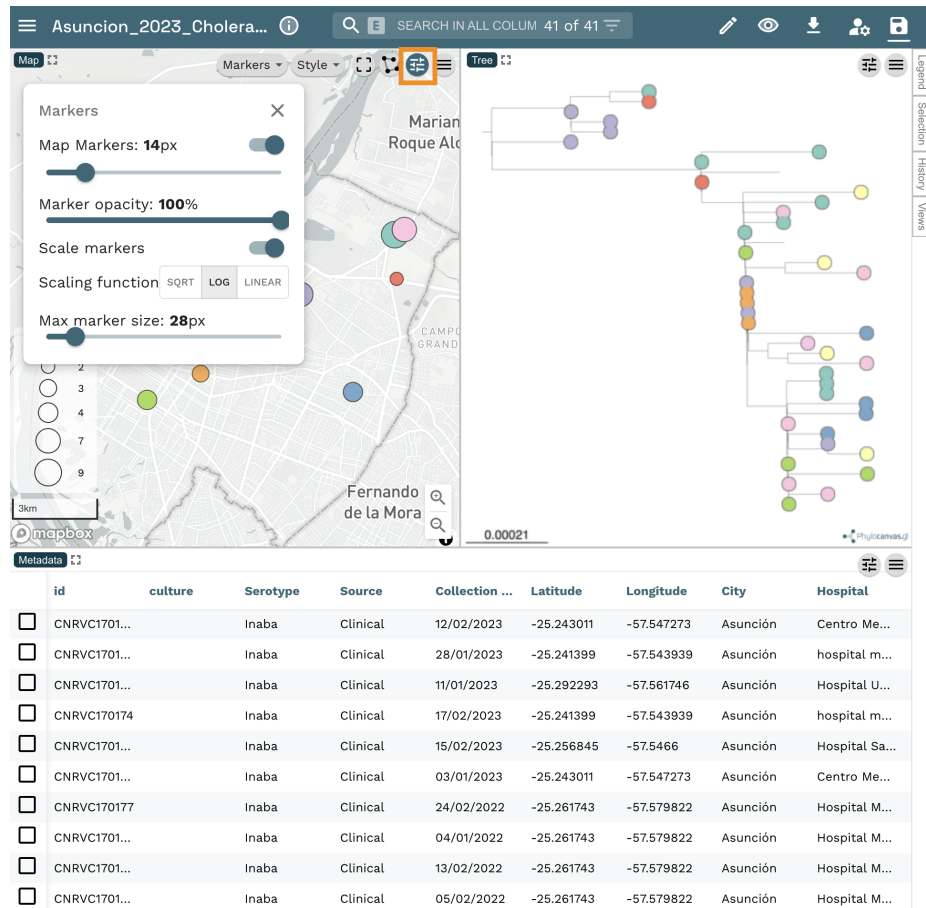
Open the Microreact link in the output from data-flo. It should look something like this:



Now change the colour column to “Hospital”. Click on the eye icon at the top left of the page. Select “Hospital” from the Colour Column dropdown list.



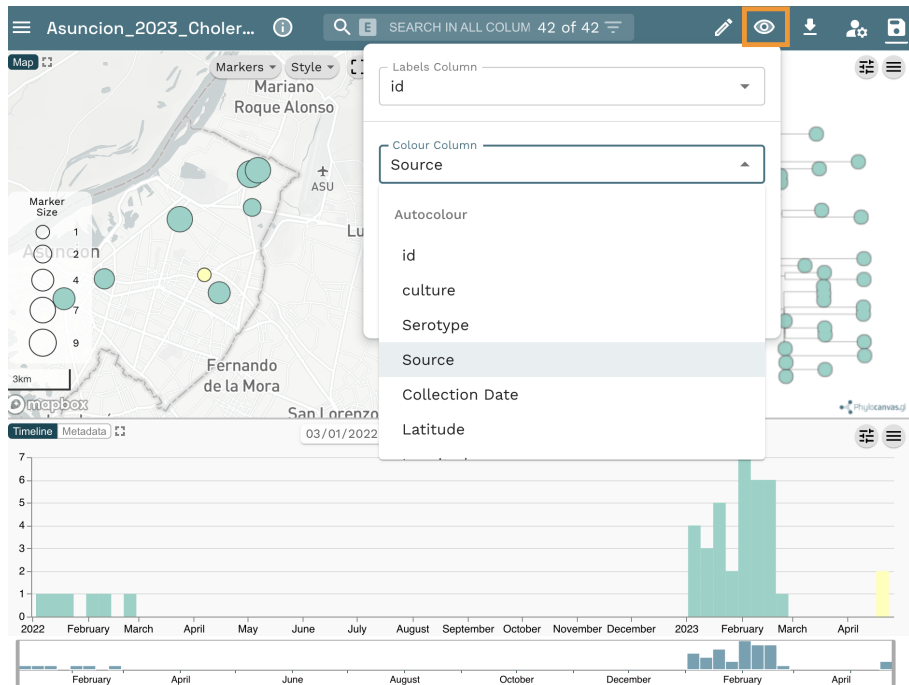
Scale the markers on the map by clicking on the sliders icon, then in the opening options click on **Markers**. Finally, toggle the **Scale markers** option on.



Answer the following questions:

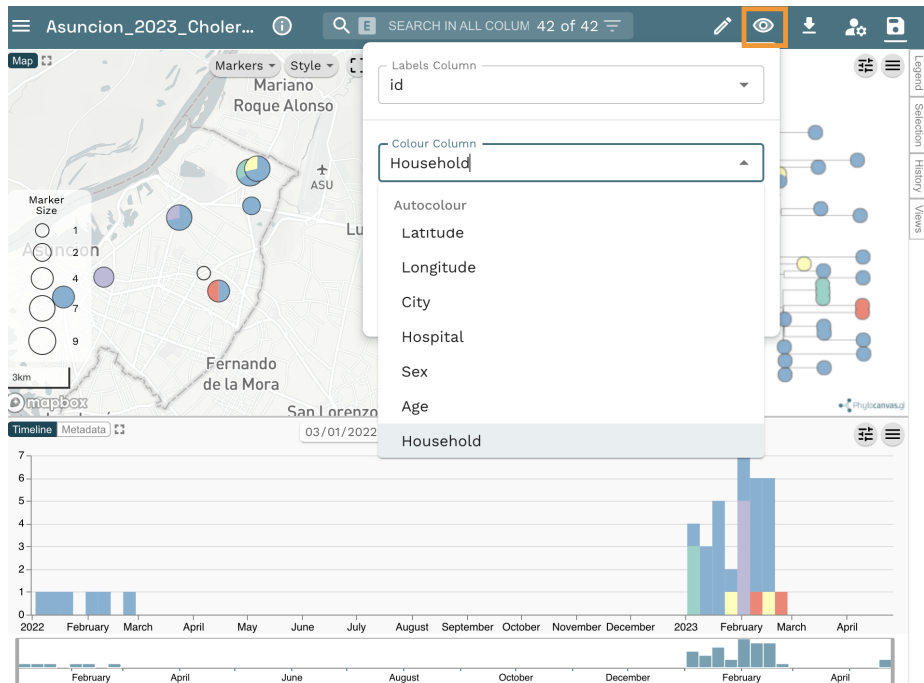
1. What do the markers on the map represent?
2. Which hospital reported the index (first) case? Tip: Click on the earliest sample on the timeline.

Change the colour column to "Source". Click on the eye icon at the top left of the page. Then select "Source" from the Colour Column dropdown list.

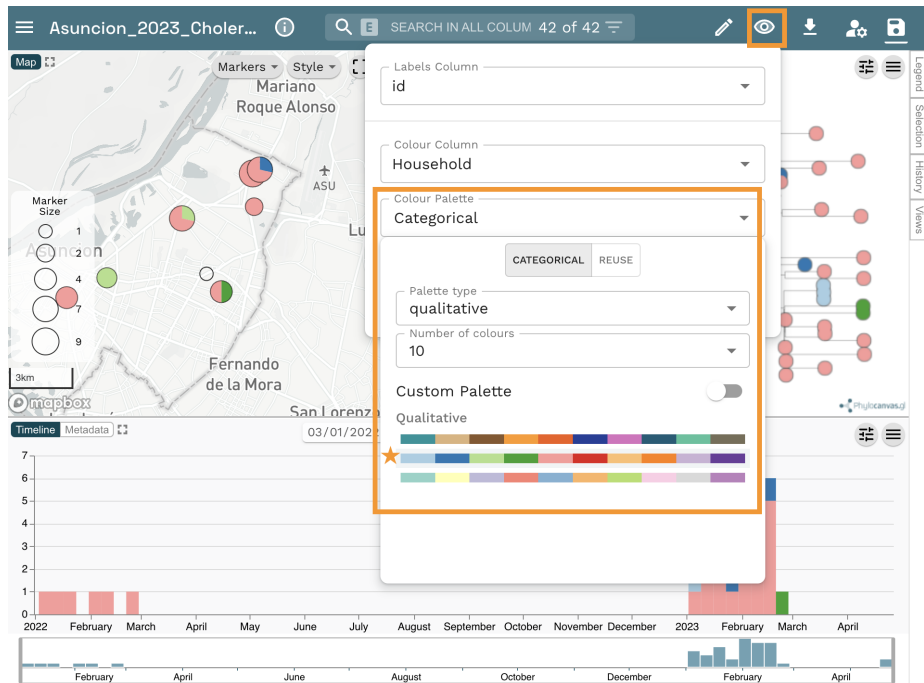


3. Can you confirm that the source of the outbreak is the contaminated water from Ñu Guasú Park?
4. Why is it useful to include genomes from a previous outbreak in the analysis? Is the current outbreak related to the past outbreak from Jan-Feb 2020?

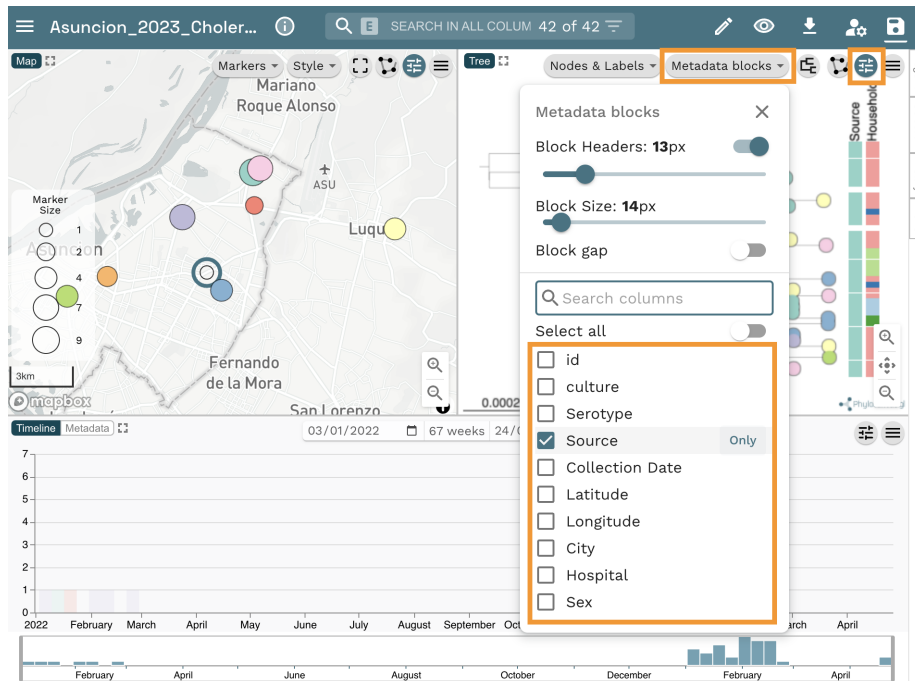
Microreact assigns colours automatically to every column in your metadata table, but you can customise them. Let's change the colour column to "Household". Click on the eye icon at the top left of the page. Then select "Household" from the Colour Column dropdown list.



Now Select “Categorical” from the Colour Palette option. Next Select “qualitative” from the Palette type dropdown list and “10” from the Number of colours dropdown list. Finally, select the second option from the list of palettes.



Change the colour column back to “Hospital” and add the columns “Source” and “Household” as metadata blocks. For this, select the slider icon from top right of the map panel, then, click on the **Metadata blocks** button. Finally, select “Source” and “Household” from the list of options.



5. Do any of the cases share a household? Taking into account the incubation period for cholera (a few hours to five days), does the data support intra-household transmission?

## Resources

### Install and test Epicollect5 on your mobile phone

Epicollect5 is available on Android and iOS. To install it on your mobile device, get it from Google Play or the Apple App Store. To test Epicollect5 on your phone, open the application and click on the pre-loaded EC5 Demo Project. Click on + Add entry and follow the questionnaire. Please answer all the questions, including taking a photo with your phone's camera. Once you've answered the questions, save and upload the entry. Please note that you must be connected to the internet (or to a mobile network) to be able to upload an entry.

### Sign-in to data-flo


<https://docs.data-flo.io/introduction/getting-started-sign-in>

### Sign-in to Microreact

Head over to <https://microreact.org/my-account>, and sign up using your email address or any other authenticating option





**Email**  
   
Sign in with Email  

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or

  
Sign in with Google  
  
Sign in with Facebook  
  
Sign in with Twitter (Legacy)

## Web colours

Web colours are used on web pages, such as the microreact.org projects, and are usually specified in hexadecimal format preceded by a number sign (or hashtag sign if you were born before the Jurassic period). For example, #FFFFFF is the

hexadecimal code (or hex code) for the colour “white”, #000000 is the hex code for the colour “black”, and #FF00FF is the hex code for the colour “magenta”. For more information on the format of the hexadecimal code see Wikipedia page [https://en.wikipedia.org/wiki/Web\\_colors](https://en.wikipedia.org/wiki/Web_colors)

Useful websites to work with web colours:

### **ColorBrewer**

<https://colorbrewer2.org/#type=sequential&scheme=BuGn&n=3>  
Colour palettes for maps

### **ColorHexa**

<https://www.colorhexa.com/> Get complementary colours, colour gradients, etc.

### **Coolors**

<https://coolors.co/palettes/trending> Colour palette generator

### **Wes Anderson colour palettes**

<https://github.com/karthik/wesanderson> Hipster colour palettes inspired by Wes Anderson movies

## **Bonus activities**

1. Download data from the Epicollect5 project on the web.
  - a. Go to the [epicollect](#) link provided above.
  - b. Select VIEW DATA.

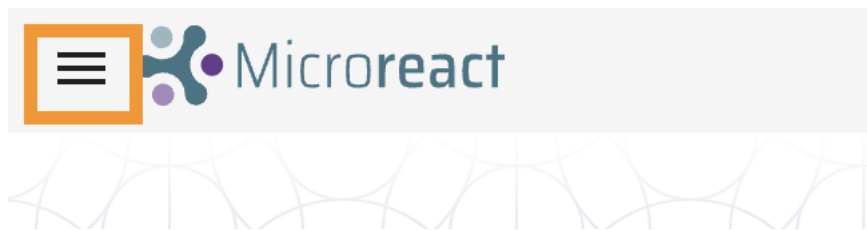


c. Click on **Download** and select your format of choice.

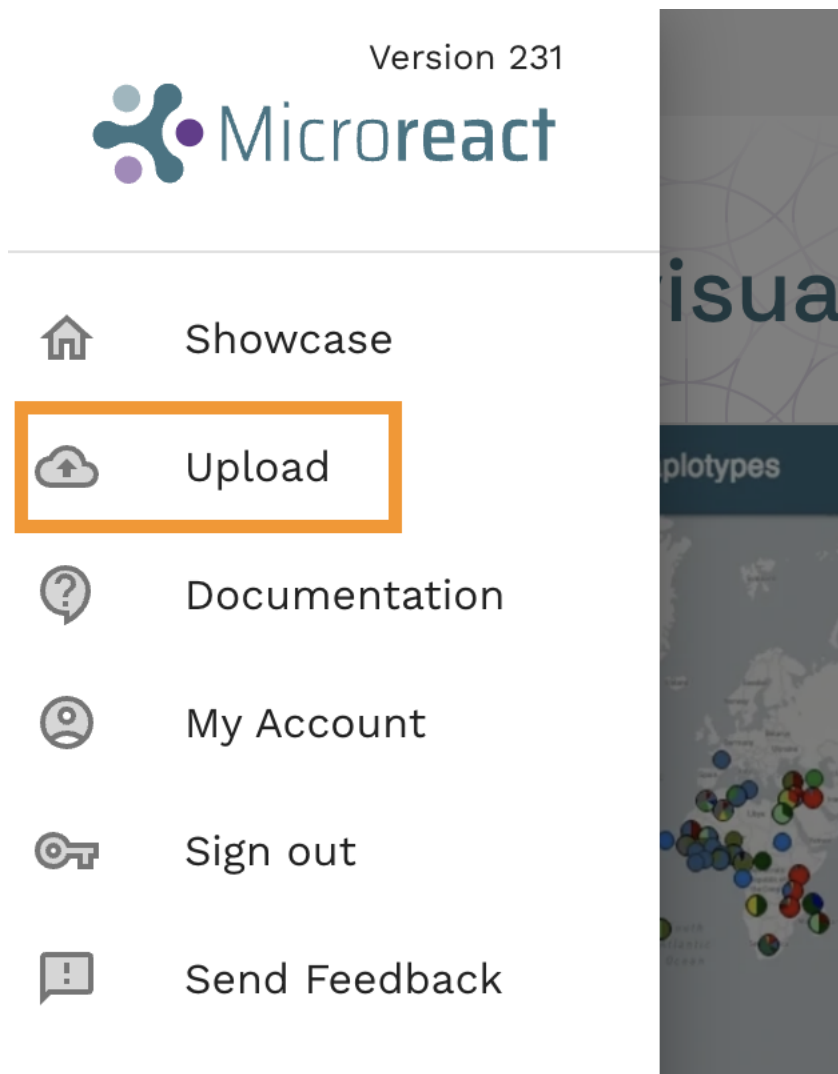
| View | Delete | Edit | Title                | Created At    | Collector's Name and Last Name | Location Type | Please describe location | Ty |
|------|--------|------|----------------------|---------------|--------------------------------|---------------|--------------------------|----|
|      |        |      | Julio Díaz Caballero | 9th Apr, 2023 | Julio Díaz Caballero           | Outdoors      |                          | Hc |

## 2. Create a microreact project using a metadata in csv format and a phylogenetic tree.

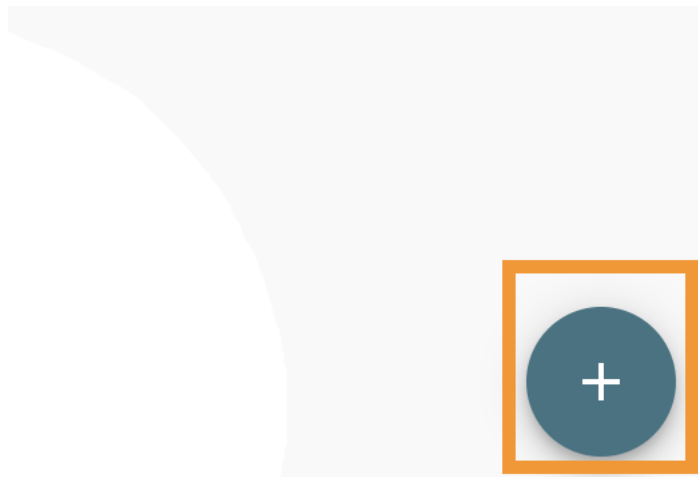
a. Click on the menu icon at the top left of the screen



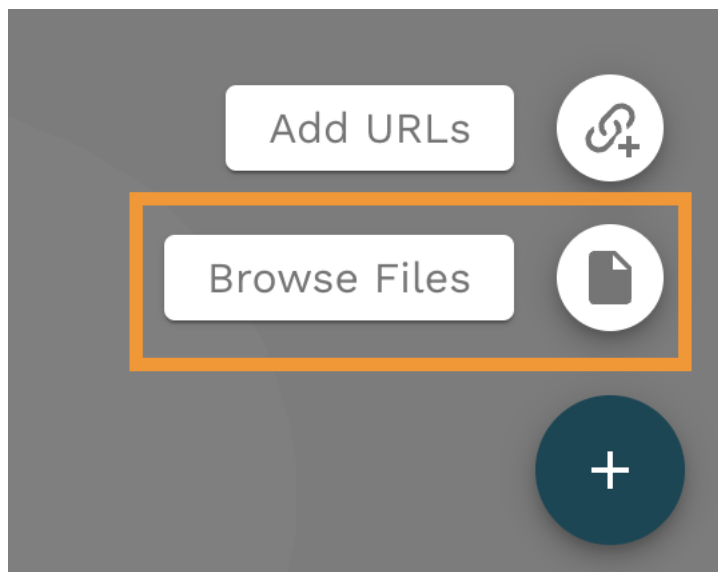
b. Click on Upload



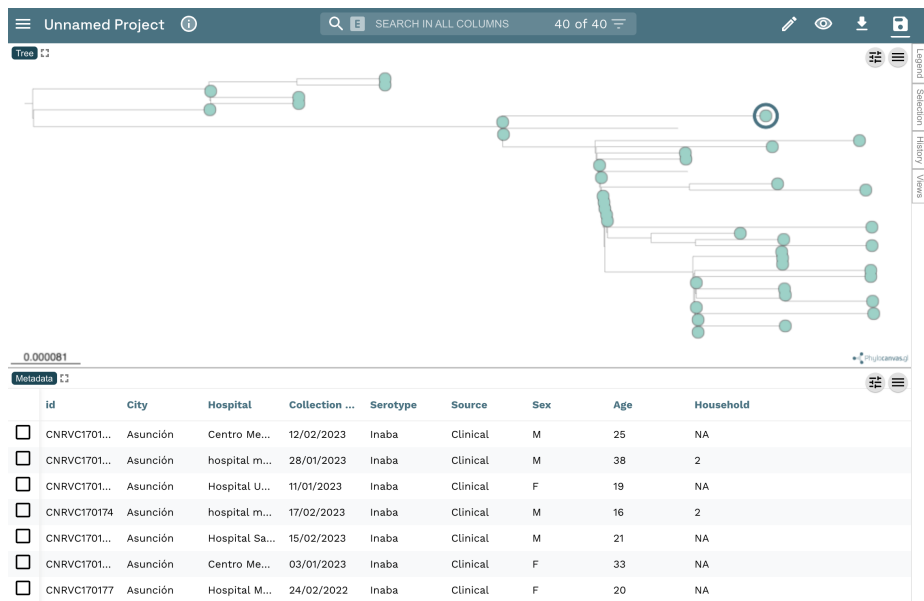
c. Click on the + symbol at the bottom right of the screen.



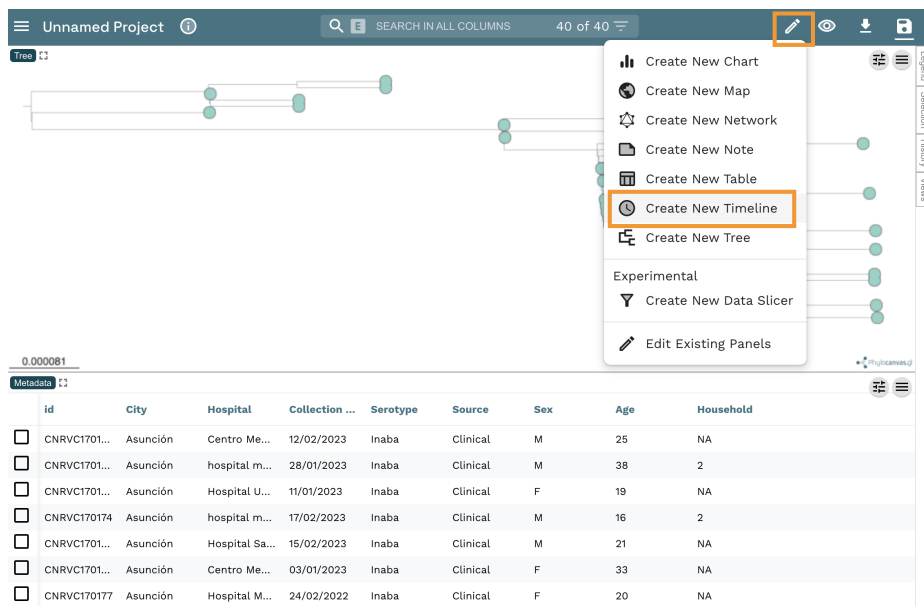
d. Select **Browse Files**



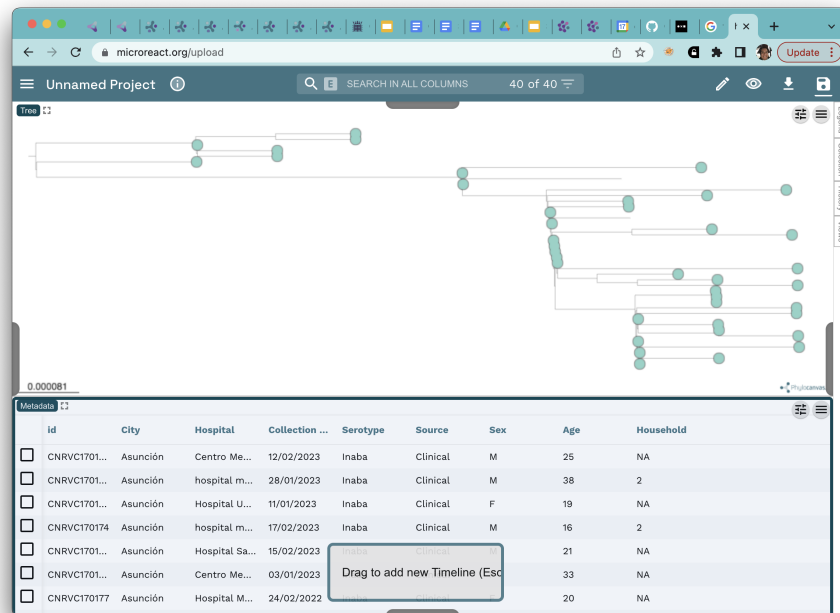
- e. Choose the files `tree.nwk` and `epi_data.csv`, and click on the **Open** button (These files were downloaded in the dataflo section).
- f. Click on **Continue** in the next prompts.
- g. Your view will look like this:



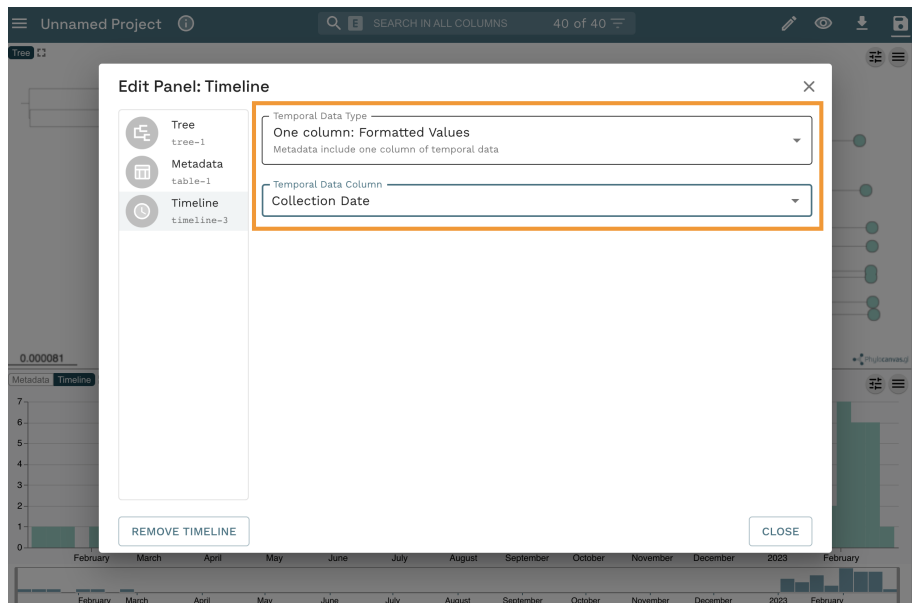
h. You can add a Timeline by clicking on the pencil icon at the top left of the creen, and clicking on **Create New Timeline**



i. Then position the new panel by dragging the pointer to the bottom panel.

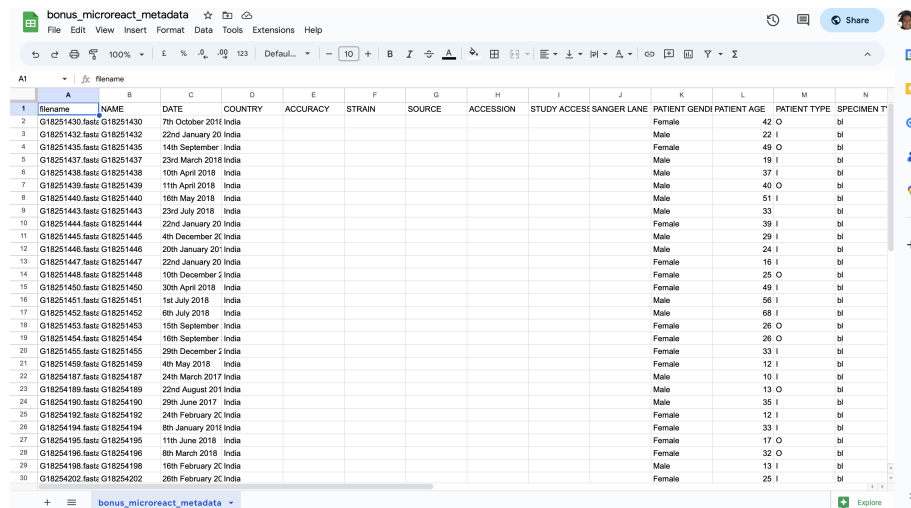


- j. Finally, select “One column: Formatted Values” from the Temporal Data Type dropdown list, and “Collection Date” from the Temporal Data Column dropdown list. Click on CLOSE



### 3. Create a microreact project from a Google Spreadsheet

#### a. Open this Google spreadsheet



| filename        | NAME      | DATE               | COUNTRY | ACCURACY | STRAIN | SOURCE | ACCESSION | STUDY | ACCESS | SANGER LANE | PATIENT GENDI | PATIENT AGE | PATIENT TYPE | SPECIMEN T |
|-----------------|-----------|--------------------|---------|----------|--------|--------|-----------|-------|--------|-------------|---------------|-------------|--------------|------------|
| G18251430.fasta | G18251430 | 7th October 2016   | India   |          |        |        |           |       |        |             | Female        | 42          | O            | bl         |
| G18251432.fasta | G18251432 | 22nd January 2017  | India   |          |        |        |           |       |        |             | Male          | 22          | I            | bl         |
| G18251435.fasta | G18251435 | 14th September     | India   |          |        |        |           |       |        |             | Female        | 49          | O            | bl         |
| G18251437.fasta | G18251437 | 23rd March 2018    | India   |          |        |        |           |       |        |             | Male          | 19          | I            | bl         |
| G18251438.fasta | G18251438 | 10th April 2018    | India   |          |        |        |           |       |        |             | Male          | 37          | I            | bl         |
| G18251439.fasta | G18251439 | 11th April 2018    | India   |          |        |        |           |       |        |             | Male          | 40          | O            | bl         |
| G18251440.fasta | G18251440 | 16th May 2018      | India   |          |        |        |           |       |        |             | Male          | 51          | I            | bl         |
| G18251443.fasta | G18251443 | 23rd July 2018     | India   |          |        |        |           |       |        |             | Male          | 33          | bl           |            |
| G18251444.fasta | G18251444 | 22nd January 2017  | India   |          |        |        |           |       |        |             | Female        | 39          | I            | bl         |
| G18251445.fasta | G18251445 | 4th December 2016  | India   |          |        |        |           |       |        |             | Male          | 29          | I            | bl         |
| G18251446.fasta | G18251446 | 20th January 2017  | India   |          |        |        |           |       |        |             | Male          | 24          | I            | bl         |
| G18251447.fasta | G18251447 | 22nd January 2017  | India   |          |        |        |           |       |        |             | Female        | 16          | I            | bl         |
| G18251448.fasta | G18251448 | 10th December 2016 | India   |          |        |        |           |       |        |             | Female        | 25          | O            | bl         |
| G18251450.fasta | G18251450 | 30th April 2018    | India   |          |        |        |           |       |        |             | Female        | 49          | I            | bl         |
| G18251451.fasta | G18251451 | 1st July 2018      | India   |          |        |        |           |       |        |             | Male          | 56          | I            | bl         |
| G18251452.fasta | G18251452 | 6th July 2018      | India   |          |        |        |           |       |        |             | Male          | 68          | I            | bl         |
| G18251453.fasta | G18251453 | 15th September     | India   |          |        |        |           |       |        |             | Female        | 26          | O            | bl         |
| G18251454.fasta | G18251454 | 16th September     | India   |          |        |        |           |       |        |             | Female        | 26          | O            | bl         |
| G18251455.fasta | G18251455 | 29th December 2016 | India   |          |        |        |           |       |        |             | Female        | 33          | I            | bl         |
| G18251459.fasta | G18251459 | 4th May 2018       | India   |          |        |        |           |       |        |             | Female        | 12          | I            | bl         |
| G18251487.fasta | G18251487 | 24th March 2017    | India   |          |        |        |           |       |        |             | Male          | 10          | I            | bl         |
| G18251489.fasta | G18251489 | 22nd August 2017   | India   |          |        |        |           |       |        |             | Male          | 13          | O            | bl         |
| G18251490.fasta | G18251490 | 29th June 2017     | India   |          |        |        |           |       |        |             | Male          | 35          | I            | bl         |
| G18251492.fasta | G18251492 | 24th February 2017 | India   |          |        |        |           |       |        |             | Female        | 12          | I            | bl         |
| G18251494.fasta | G18251494 | 8th January 2018   | India   |          |        |        |           |       |        |             | Female        | 33          | I            | bl         |
| G18251495.fasta | G18251495 | 11th June 2018     | India   |          |        |        |           |       |        |             | Female        | 17          | O            | bl         |
| G18251496.fasta | G18251496 | 8th March 2018     | India   |          |        |        |           |       |        |             | Female        | 32          | O            | bl         |
| G18251498.fasta | G18251498 | 16th February 2017 | India   |          |        |        |           |       |        |             | Male          | 13          | I            | bl         |
| G18251499.fasta | G18251499 | 16th February 2017 | India   |          |        |        |           |       |        |             | Female        | 25          | I            | bl         |

- b. Make a copy of this in your own Google account by selecting **Make a copy** from the **File** menu item. When prompted, click on **Make a copy**. This will open a separate tab where the copy will be available.



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| 25 | G18254192.fastq | G18254192 | 24th February 2018 India |
| 26 | G18254194.fastq | G18254194 | 8th January 2018 India   |
| 27 | G18254195.fastq | G18254195 | 11th June 2018 India     |
| 28 | G18254196.fastq | G18254196 | 8th March 2018 India     |
| 29 | G18254198.fastq | G18254198 | 16th February 2018 India |
| 30 | G18254202.fastq | G18254202 | 26th February 2018 India |


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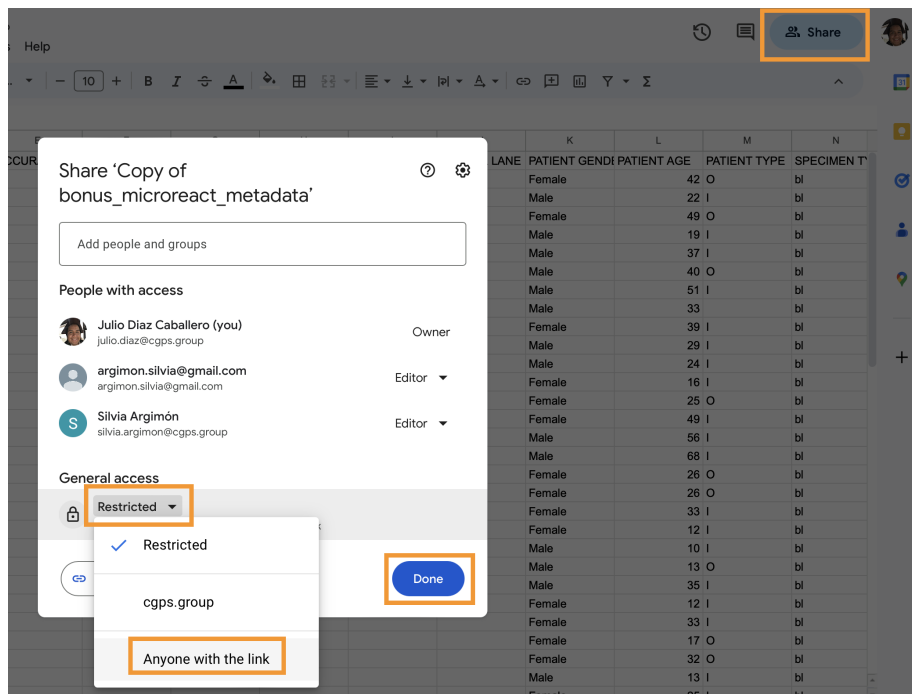
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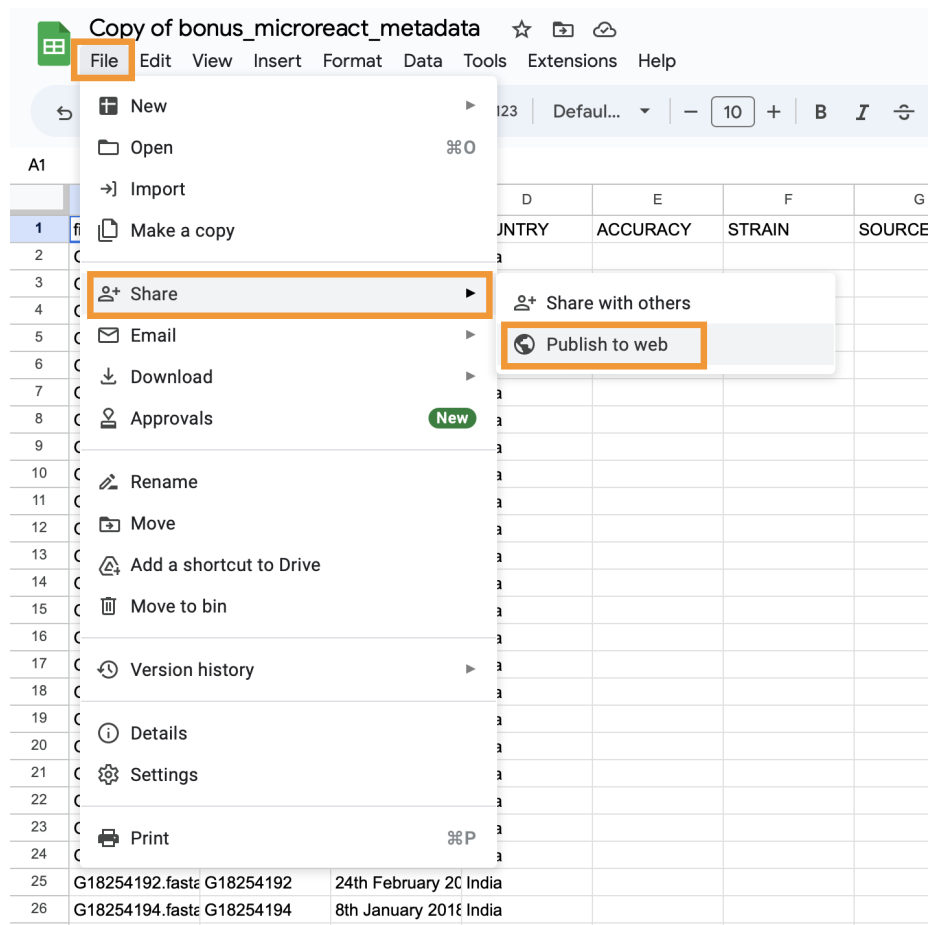
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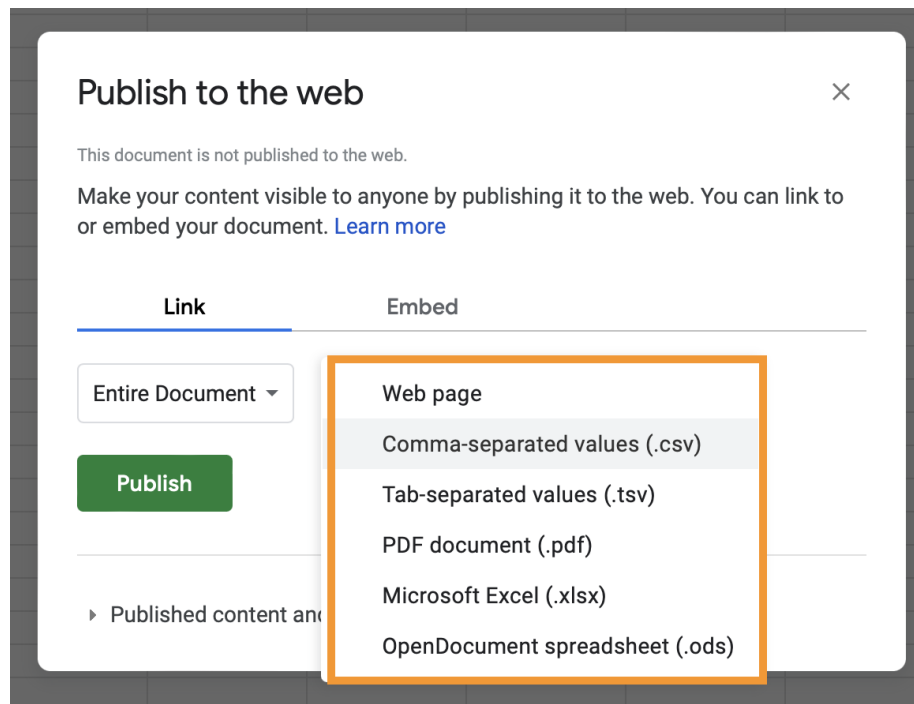
- c. Set access to shareable by clicking on the **Share** button at the top right of the screen. A dialogue screen will popup, here, click on the **Restricted** button, select **Anyone with the link**, and click on **Done**



- d. Now to publish the google spreadsheet click on the File menu item, select Share, and click on Publish on web



- e. In the popup message click on Web page, and select Comma-separated values (.csv). Also, make sure the Automatically republish when changes are made option is turned on under the Published content and settings section



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g. Go to **microreact.org**, and select Upload from the main menu.

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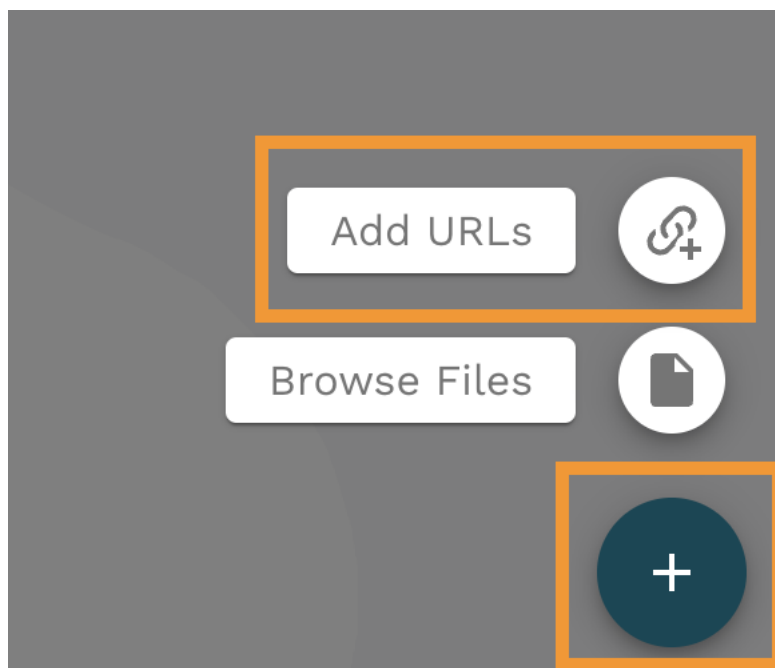
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h. Select the plus icon at the bottom right, and click on **Add URLs**





- i. Paste the url from step *f* and select **Data** (CSV or TSV) under **File kind**. Finally click on **CONTINUE** to see your microreact.

A screenshot of the 'Add Files or URLs' dialog box. The 'File' field contains the URL 'https://docs.google.com/spreadsheets/d/e/2PACX-1vS3h6VQ4ucVfLKone'. The 'File kind' dropdown is set to 'Data (CSV or TSV)'. The 'CONTINUE' button is highlighted with an orange box. The dialog also includes a 'CANCEL' button and an 'ADD MORE FILES' button.

- j. You should get a screen like this!

