

Computational Practical 12

Local Genomic Surveillance of Antimicrobial Resistance

Whole-genome sequencing (WGS) overcomes the lack of resolution of current bacterial typing methods. Several recent publications have confirmed the ability of WGS to define transmission dynamics of a single clone at different geographic and temporal scales (Alam *et al.* 2015; Aanensen *et al.* 2016; Harris *et al.* 2010, 2013; Köser *et al.* 2012). This has identified global and local transmission routes and, when combined with epidemiological data, can confirm or refute putative outbreaks.

Genomic epidemiology is changing the practice of surveillance and outbreak investigation of nosocomial and other pathogens. Genomic surveillance is becoming increasingly high profile as a mechanism to understand the spread of multidrug resistant pathogens.

Within this practical you will visualize and interpret the datasets of key studies published on the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA). We will focus on this bacterium to exemplify different applications of WGS. We will make use of Microreact (<https://Microreact.org/>), a web application that provides an interactive visualization of datasets via phylogenetic trees, maps, timelines and tables.

12.1 Hospital transmission of MRSA

12.1.1 Introduction

Whole-genome sequencing of MRSA has been used to define phylogeny and transmission in healthcare settings. Here, we will investigate the genetic diversity of MRSA within wards and individual patients in a hospital in Thailand and identify the source of numerous transmission events. All patients in two intensive care units were screened for MRSA carriage over a 3-months period. All MRSA belonged to multi-locus sequence type 239 (ST 239). A total of 79 isolates from 46 patients and five members of staff, including the first MRSA-positive screen isolates and up to two repeat isolates where available (Tong *et al.* 2015).

12.1.2 Available data

In this section, we will make use of a maximum likelihood phylogenetic tree of the 171 ST239 isolates (Tong2015_tree.nwk) and a metadata file with information on the patient Id, ward, time of isolation and antibiotic resistance phenotypes (Tong2015_metadata.csv).

12.1.3 Creating a Microreact project

Start by opening up a new window in Firefox and typing <https://Microreact.org/> in the address bar. Click on “Upload” as shown in Figure 12.1 to create a new project.

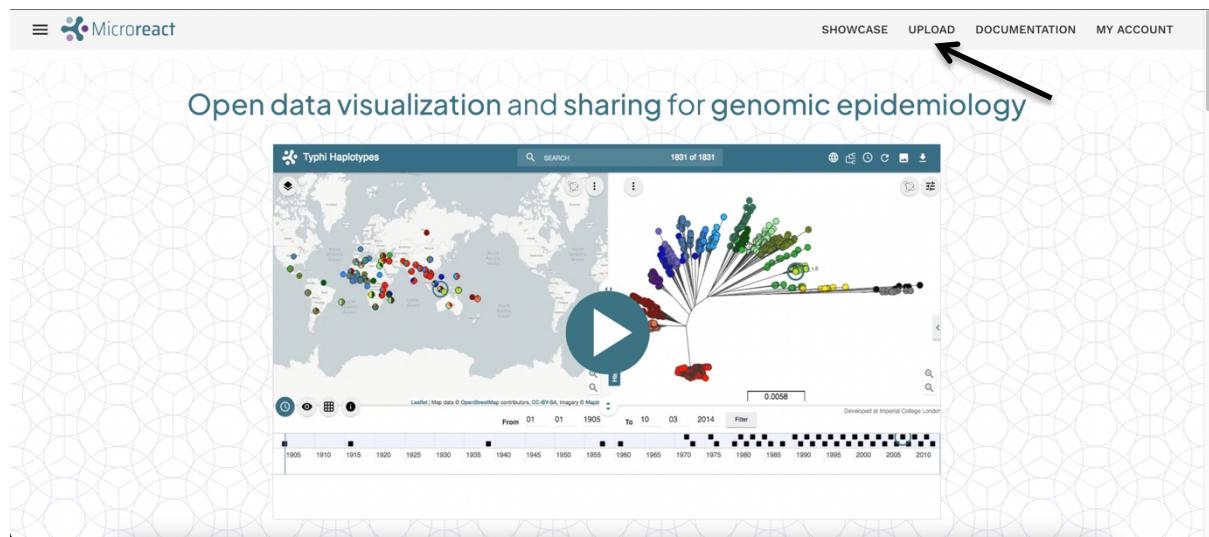


Figure 12.1 Microreact home page

Drag and drop files “Tong2015_metadata.csv” and “Tong2015_tree.nwk” from your file browser onto your Internet browser (Figure 12.2).

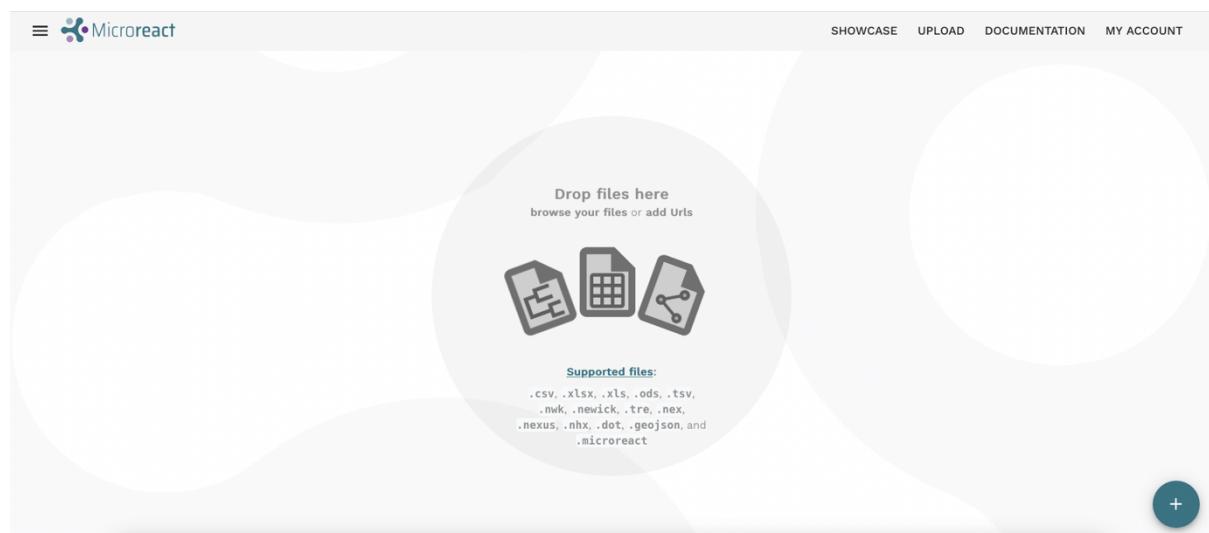


Figure 12.2 Microreact upload page

Once the tree and metadata files are loaded you will be directed to a new window where files will be automatically detected as Data (CSV or TSV) file (Tong2015_metadata.csv) and Tree (Newick) file (Tong2015_tree.nwk). In this new window click on ‘Continue’. In the next window (Figure 12.3), make sure column ‘id’ is selected as the ‘ID column’ and then click on ‘Continue’.

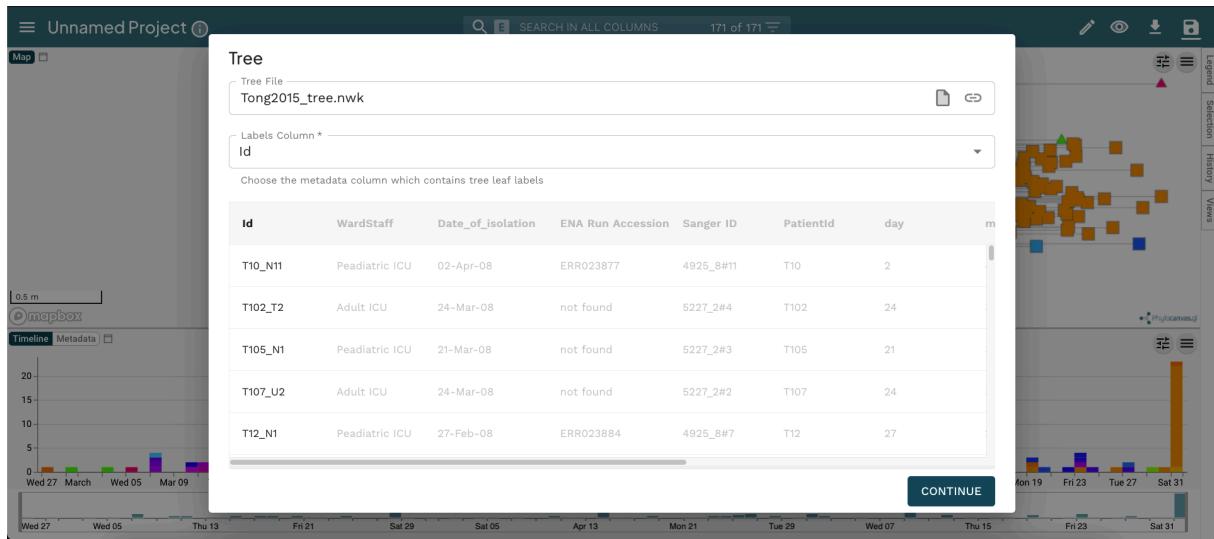


Figure 12.3 Data Table window in Microreact

As the country of origin is irrelevant in this investigation (because all isolates come from the same country and hospital) click on the ‘add or edit panels’ button at the top-right corner of the screen (arrow 1 in Figure 12.4), and then on ‘Edit Existing Panels’ (arrow 2).



Figure 12.4 Editing existing panels in Microreact

In the newly opened window (Figure 12.5), click on ‘Map’ and ‘Remove Map’ to hide the Map panel and allow more space for the phylogenetic tree.

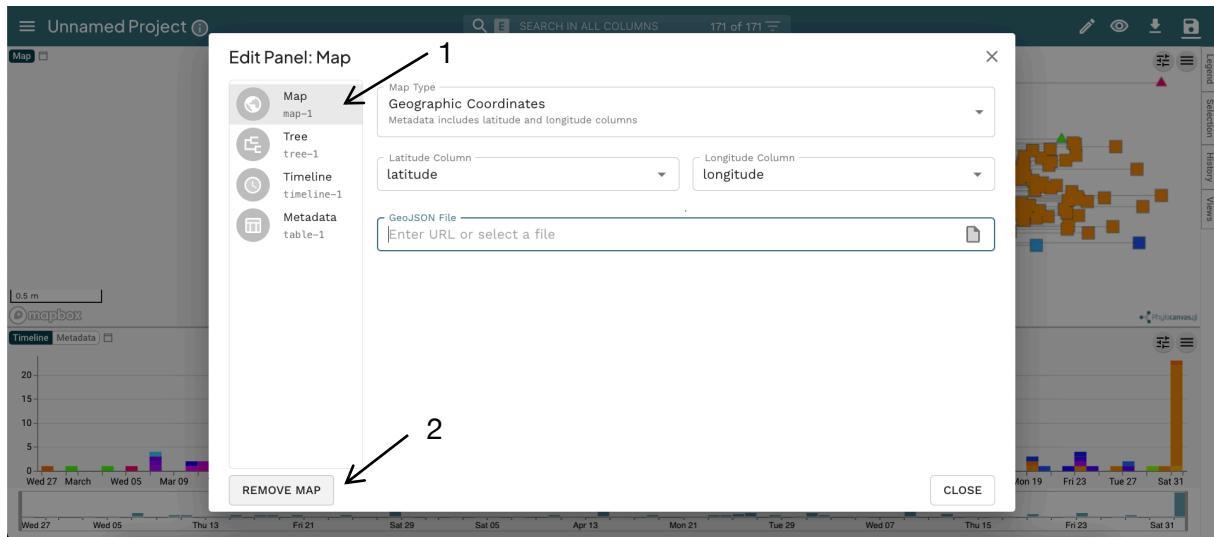


Figure 12.5 Removing Map panel in Microreact

Click on the configuration button (arrow in Figure 12.6) and under 'Tree Type' select 'Hierarchical' .



Figure 12.6 Changing phylogenetic tree layout to hierarchical

Note that colours in the tips of the tree represent different hosts, whereas shapes represent the type of host: adult patients are represented by squares, paediatric patients as triangles and nurses as circles (Figure 12.6). 1

Do you spot any clustering by ward? in other words, is there any evidence of ward-specific clades? Is there evidence of intra-ward transmission and/or inter-ward transmission?

There is evidence of intra-ward transmission revealed by the phylogenetic clustering of paediatric patients in one clade (defined by node 29, dotted red rectangle in Figure 12.6) and adult patients in another separate clade (defined by node 109, dotted blue

rectangle). There is also evidence of inter-ward transmission revealed by clades containing both paediatric and adult patients (e.g. those defined by nodes 6 and 13).

Click on the ‘Legend’ button at the right-hand side of the window (arrow 1 in Figure 12.7). A list of patient IDs will be displayed with a colour legend. Click on different patient IDs to highlight all isolates from the same patient in the tree and timeline panels as shown in Figure 12.7 for patient T126 (arrow 2).



Figure 12.7 Isolates from patient T126 are highlighted in the tree and timeline

Now, we are going to investigate cases of patient-to-patient transmission within the same ward. The most obvious example is that of patient T126 who had multiple isolates of their bacterial clone sequenced over time as highlighted in Figure 12.7. The fact that all isolates from T126 belong to the same monophyletic clade with multiple variants (i.e. branches originating from the same internal node) points to the presence of a genetic ‘cloud of diversity’ as opposed to colonisation with multiple clones (isolates present in different phylogenetic clades).

Try to identify patients with multiple isolates in the tree. Specifically, compare patients T20, T35, T99, T126, T188 and T234. From these, try to differentiate patient colonised with multiple bacterial clones (mixed colonisation) from patients with variants of the same clone (also refer as to ‘cloud of diversity’). Patients T126, T188 or T20 have evidence of a cloud of diversity whereas T234, T99 or T35 have evidence of multiple clones (mixed colonisation).

Comparison of isolates from patient T126 with isolates from other patients within the same phylogenetic clade (i.e. T327, T192, T301, T234, T303, T271, T335, T232) provides evidence that T126 acted as the donor for MRSA transmission to these other patients, who were colonized with isolates derived from variants within the T126 cloud. In other words, the fact that other patients are nested within the diversity of patient T126 in the tree supports the hypothesis that T126 acted as a source.

The  icon on the top-right corner of the window (arrow 1 in Figure 12.8) will allow you to colour and label the tips of the tree based on different metadata fields. Here, in the drop-down list under 'Labels Column' select 'PatientId' to label the tips (or leaves) based on this field. Tip nodes are already colour-coded by PatientId too.

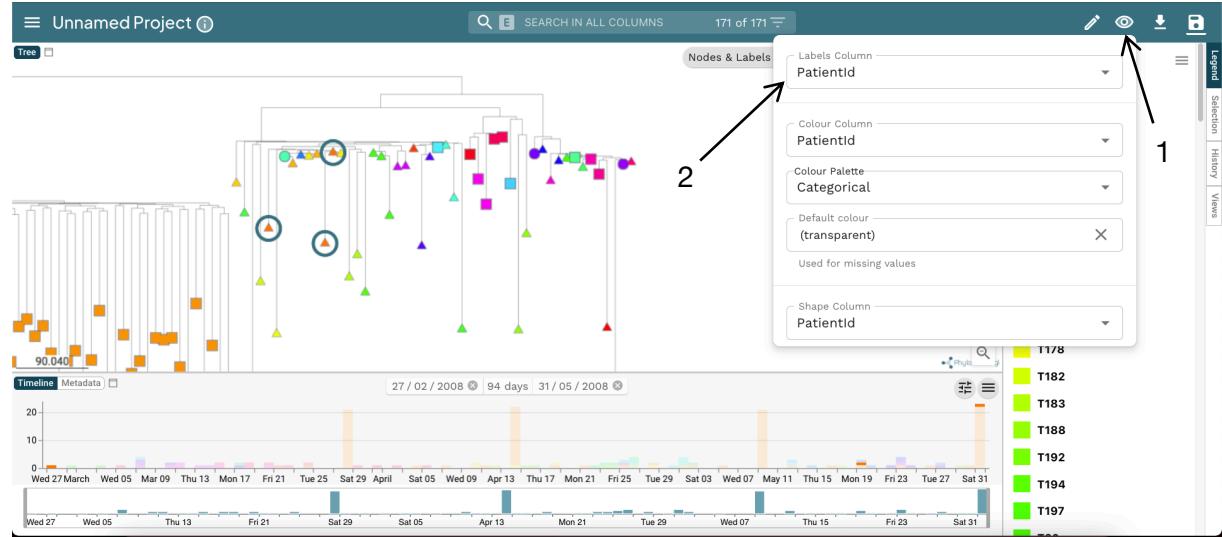


Figure 12.8 Labelling tips with patient ids

Next, browse to the part of the tree where the isolates of most paediatric case cluster. Make sure tip labels (i.e. patient ids) are visible by clicking on the 'Leaf Labels' toggle button  (arrow 2, Figure 12.9) under the 'Nodes & Labels' window (arrow 1). Highlight isolates from the following paediatric patients with more than one isolate in the tree: T12, T183 and T188 (arrow 3).

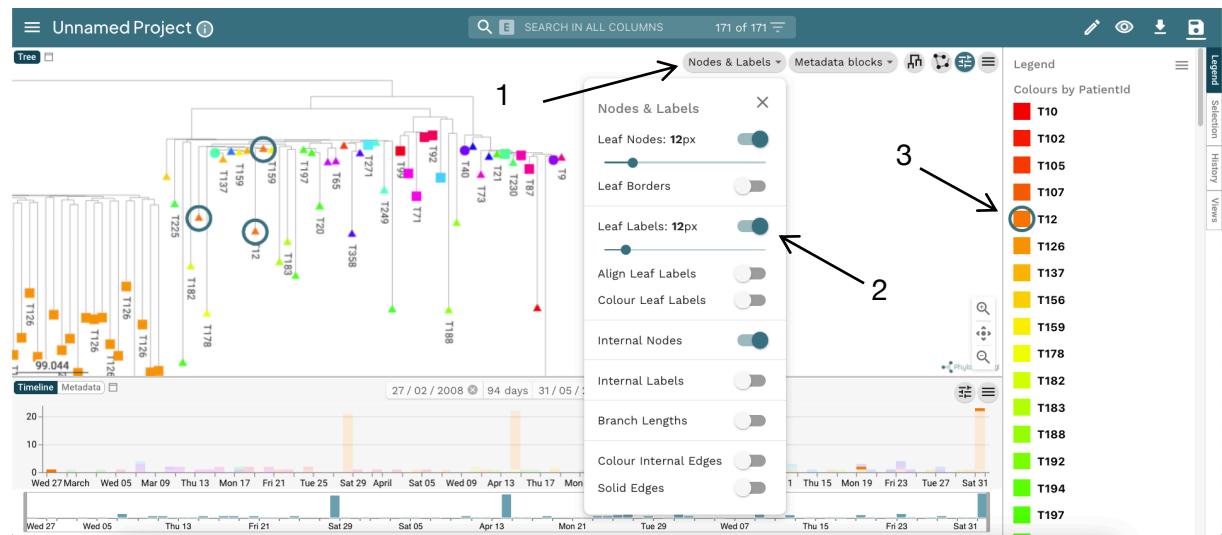


Figure 12.9 Investigation intra-patient diversity in paediatric patients

Is there any evidence of any of these patients acting as a donor in a transmission event? A group of six patients seem to have acquired their strain from patient T12 (see clade defined by node 10). Patients T183 and T188 do not have evidence of onward transmission.

12.2 Zoonotic transmission of MRSA

12.2.1 Introduction

Several methicillin-resistant *Staphylococcus aureus* (MRSA) lineages that carry a novel *mecA* homologue (*mecC*) have recently been described in livestock and humans. In Denmark, two independent human cases of *mecC*-MRSA infection were linked to a livestock reservoir. We will investigate the molecular epidemiology of the associated MRSA isolates using whole genome sequencing and assess the evidence for zoonotic transmission. Single nucleotide polymorphisms (SNP) were defined and compared to a reference genome to place the isolates into a phylogenetic context. A total of seven sequence type 130 isolates from two different patients and different livestock from two different farms where available (Harrison *et al.* 2013).

12.2.2 Available data

In this section we will make use of a maximum likelihood phylogenetic tree of the 7 ST130 isolates (Harrison2013_tree.nwk) and a metadata file with information on the host Id, farm, time of isolation and antibiotic resistance phenotypes (Harrison2013_metadata.csv).

12.2.3 Creating a Microreact project

Start by opening up a new window in Firefox and typing <https://Microreact.org> in the address bar. Click on “Upload” as shown in Figure 12.10 to create a new project.

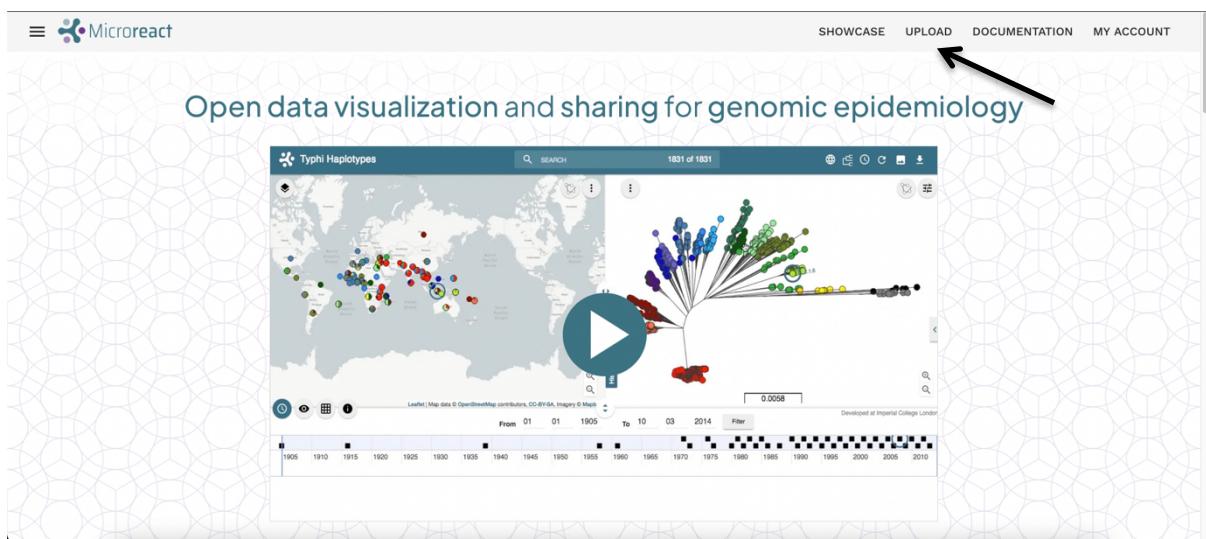


Figure 12.10 Microreact home page

Drag and drop files “Harrison2013_metadata.csv” and “Harrison2013_tree.nwk” from your file browser onto your Internet browser (Figure 12.11).

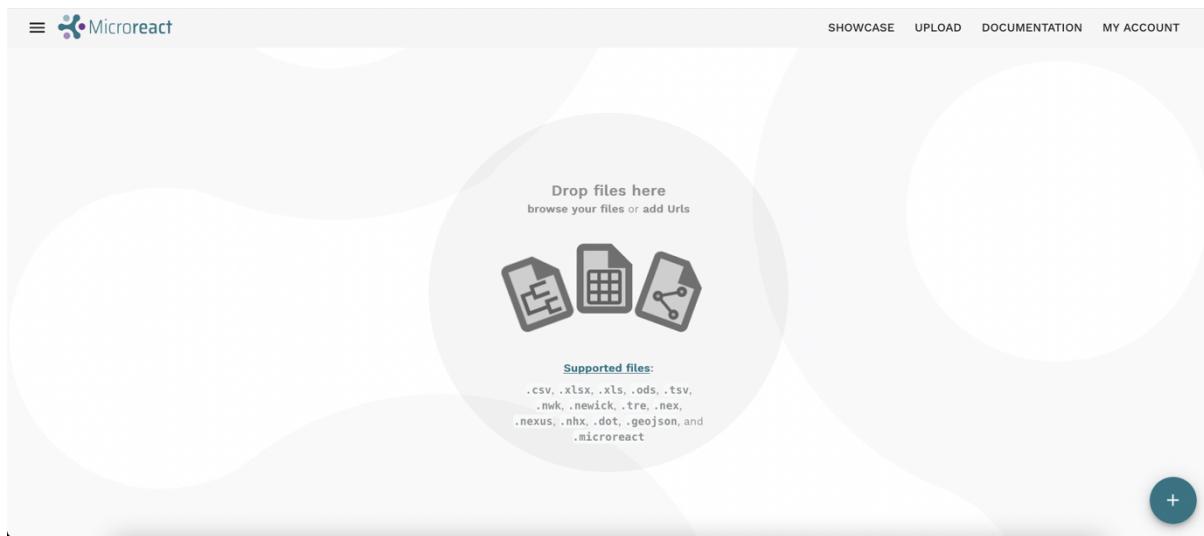


Figure 12.11 Microreact upload page

Once the tree and metadata files are loaded you will be directed to a new window where files will be automatically detected as Data (CSV or TSV) file (Harrison2013_metadata.csv) and Tree (Newick) file (Harrison2013_tree.nwk). In this new window (Figure 12.12) click on 'Continue'.

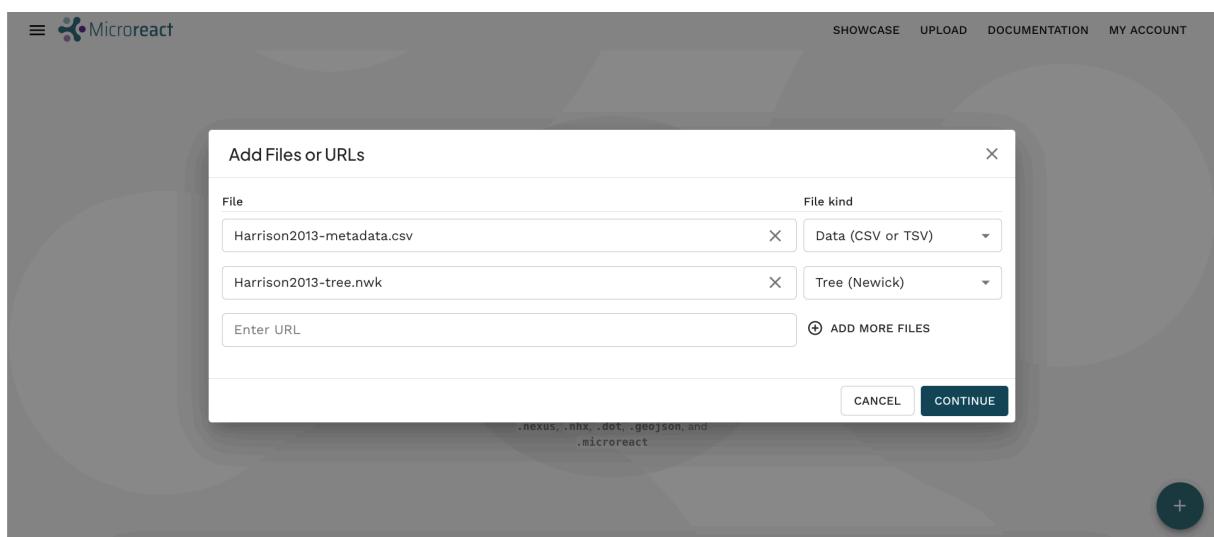


Figure 12.12 Adding files to create a Microreact project

In the next window (Figure 12.13), make sure column 'id' is selected as the 'ID column' and then click on 'Continue'.

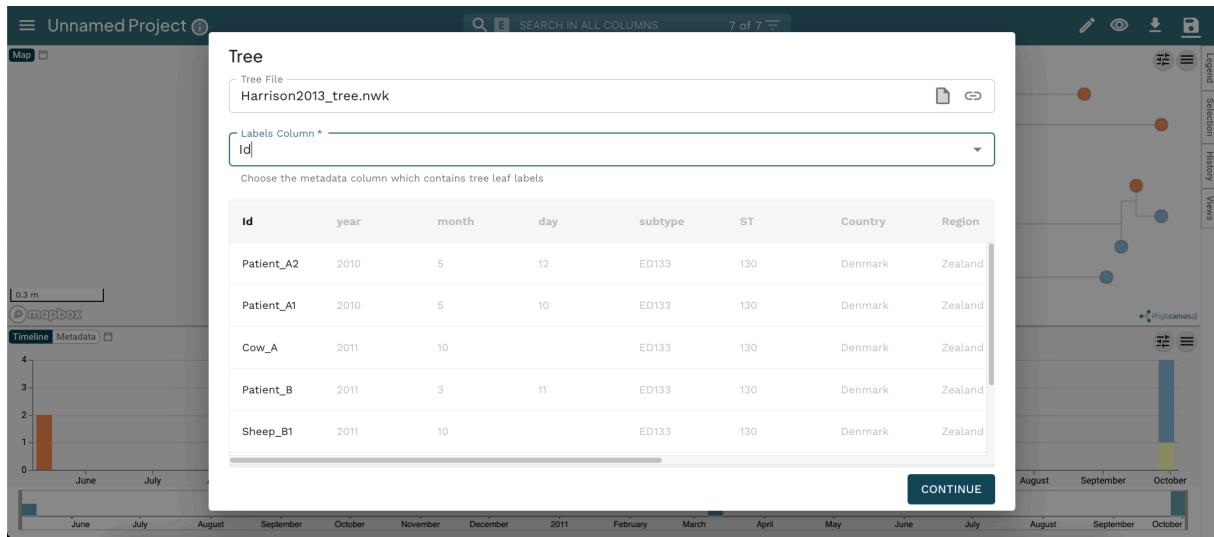


Figure 12.13 Data Table window in Microreact

Once these forms are completed your data will be utilized to create a Microreact project. You should now have a view like to the one in Figure 12.14.

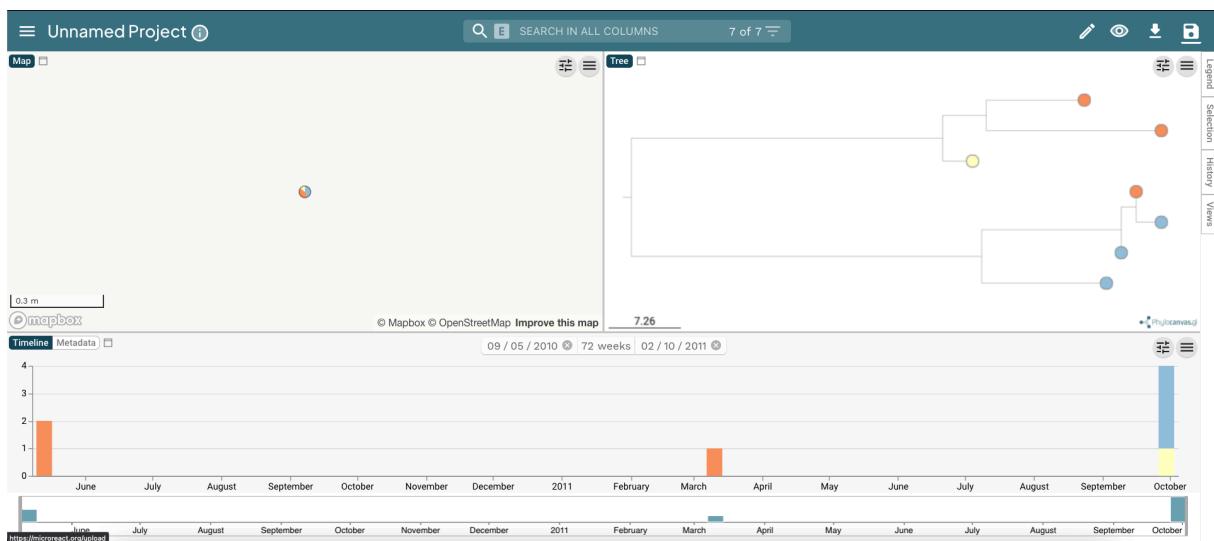


Figure 12.14 Harrison 2013 Microreact project

By default, tip nodes are coloured-coded by host (human, cow or sheep). Make sure the ‘Leaf labels’ toggle button is on under ‘Nodes & Labels’ to display sample ids, which also include the type of host and host id (Figure 12.15).

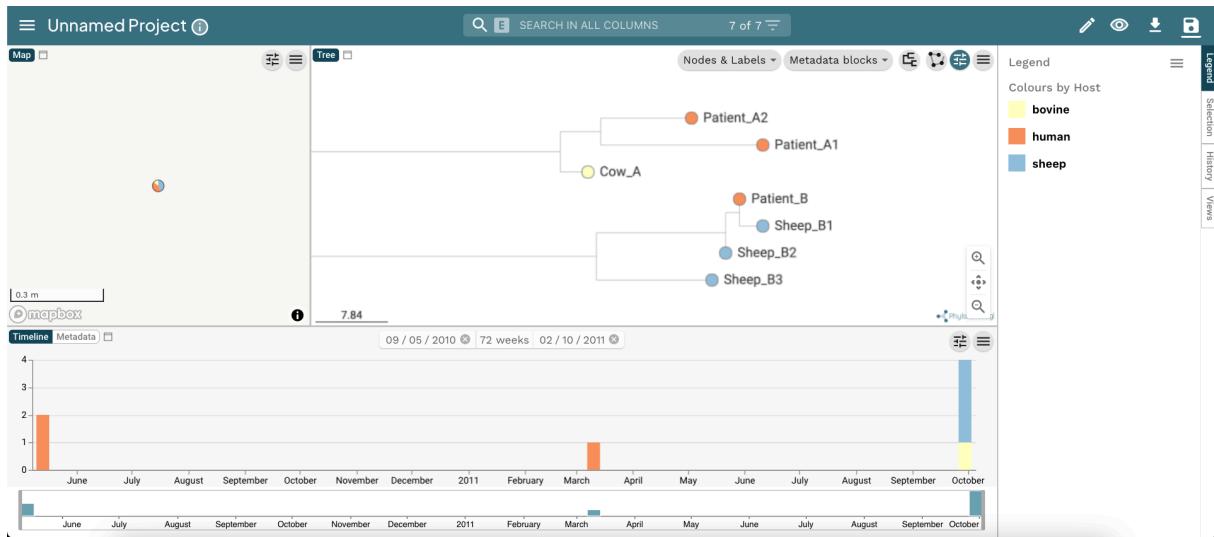


Figure 12.15 Rectangular phylogenetic view of the Harrison 2013 dataset

Now click on the ‘Labels, Colors and Shapes’ icon on the top-right corner of the window (arrow 1 in Figure 12.16). Here, in the drop-down list under ‘Labels Column’, select ‘Farm’ to label samples based on their farm of origin (arrow 2).

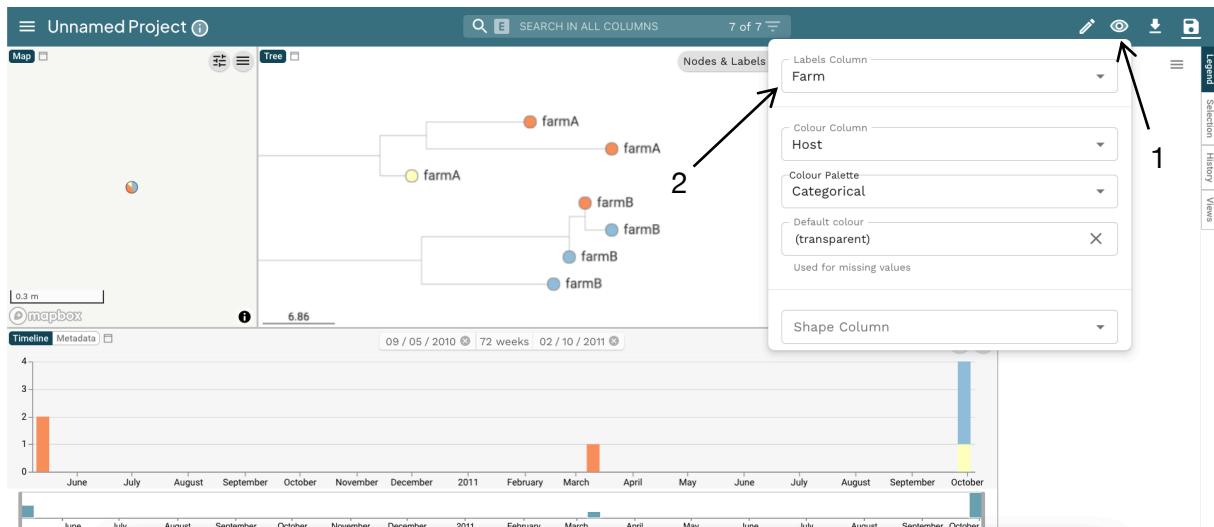


Figure 12.16 Tip nodes are labelled by farm of origin

Based on the distribution of farm ids in the phylogeny, and phylogenetic clustering of samples, is there any evidence of transmission within the same farm? Phylogenetic clustering clearly reveals two distinct farm-specific clades comprising isolates from the human case and their own livestock suggesting transmission within the same farms.

In which direction could transmission be inferred? Animal-to-human or human-to-animal? Human and animal isolates from the same farm are highly related, which supports the likelihood of zoonotic transmission. More importantly, animal isolates are at a more basal position than human and enclose (in the case of farm B) human isolates supporting an animal source, i.e. animal-to-human transmission. This study

demonstrates that *mecC*-MRSA ST130 isolates are capable of transmission between animals and humans.

References

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