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Conquering Constella: A Journey of Phage Discovery

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Conquering Constella: A Journey of Phage Discovery

Megan Frederick, Lilia Garcia, Rosemary Josenkoski, Dr. Richard Alvey

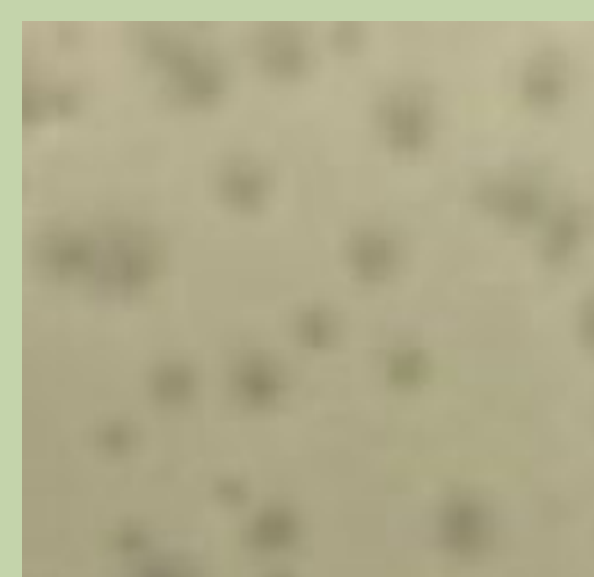
Illinois Wesleyan University Bloomington, IL

Introduction:

Bacteriophages are viruses that infect bacteria, with an estimated 10^{31} bacteriophages in existence today. Although numerous, bacteriophages have traditionally been difficult to isolate, study, and categorize. As members of the SEA-PHAGES program, we attempted to find unique phages, extract their DNA, and characterize their genomes. The name of the *Mycobacterium smegmatis* phage we chose to study was named Constella, which was isolated by Julie Xu in Bloomington, IL. With Constella, we then worked on understanding how unique this particular phage was by comparing it to other phages, both those discovered by our class as well as those with sequenced genomes. The comparison between phages was done via methods that included immunity testing, polymerase chain reaction, and transmission electron microscopy. To definitively group the phages into a cluster, we then sent them to the University of Pittsburgh for genomic sequencing. Once clustered, we used DNA annotation technology, including the programs DNAMaster and PECAAN.

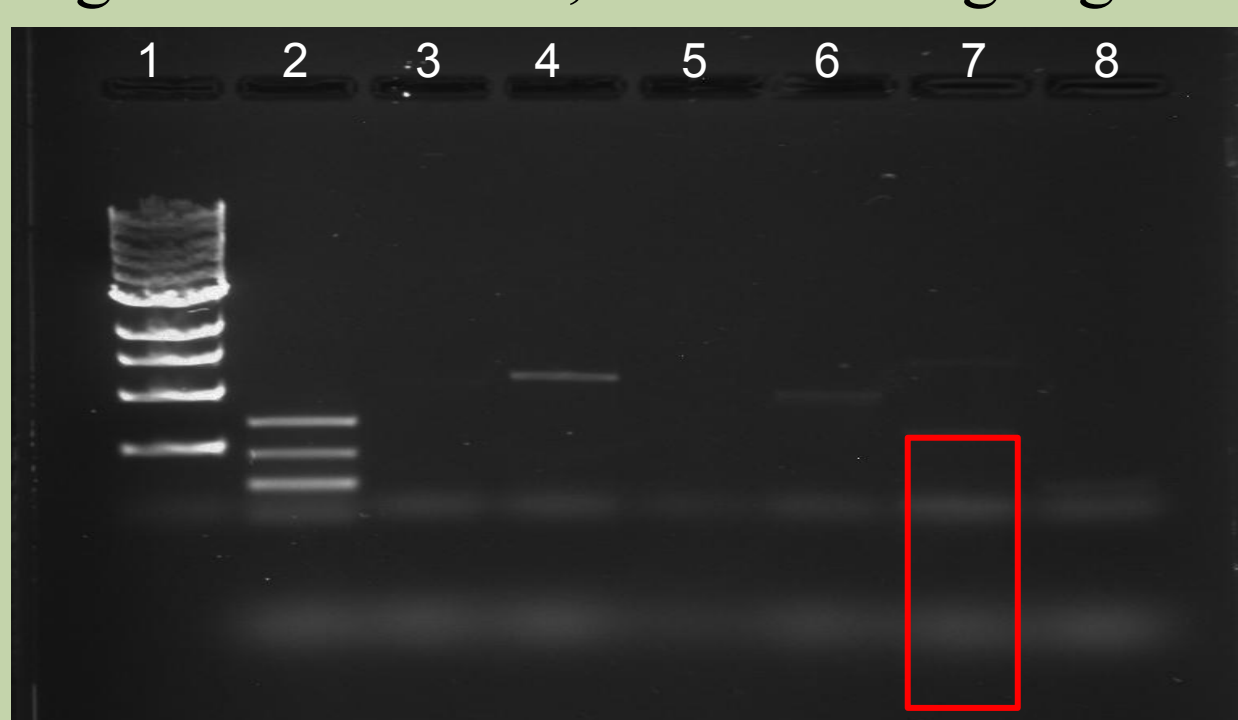
Isolation Location & Plaque Morphology:

- Constitution Trail
- Bloomington, IL
- 40.486389 N, 88.982222 W
- Dry and crumbly soil texture
- Air temperature: 25.5 °C
- Depth: 3.5 cm
- Plaques small and turbid



Polymerase Chain Reaction (PCR):

Figure 1: PCR test, Constella highlighted



- 1-Ladder
- 2-Rolex
- 3-Morrison
- 4-Silver
- 5-Tiaa
- 6-Dyad
- 7-Constella
- 8-Fright

A PCR test was done in order to attempt to cluster Constella. In this PCR where the F1 primers were used, Constella and the primer shared bands, as shown by the box. A sharing of bands is a possible indication that Constella may be a F1 phage.

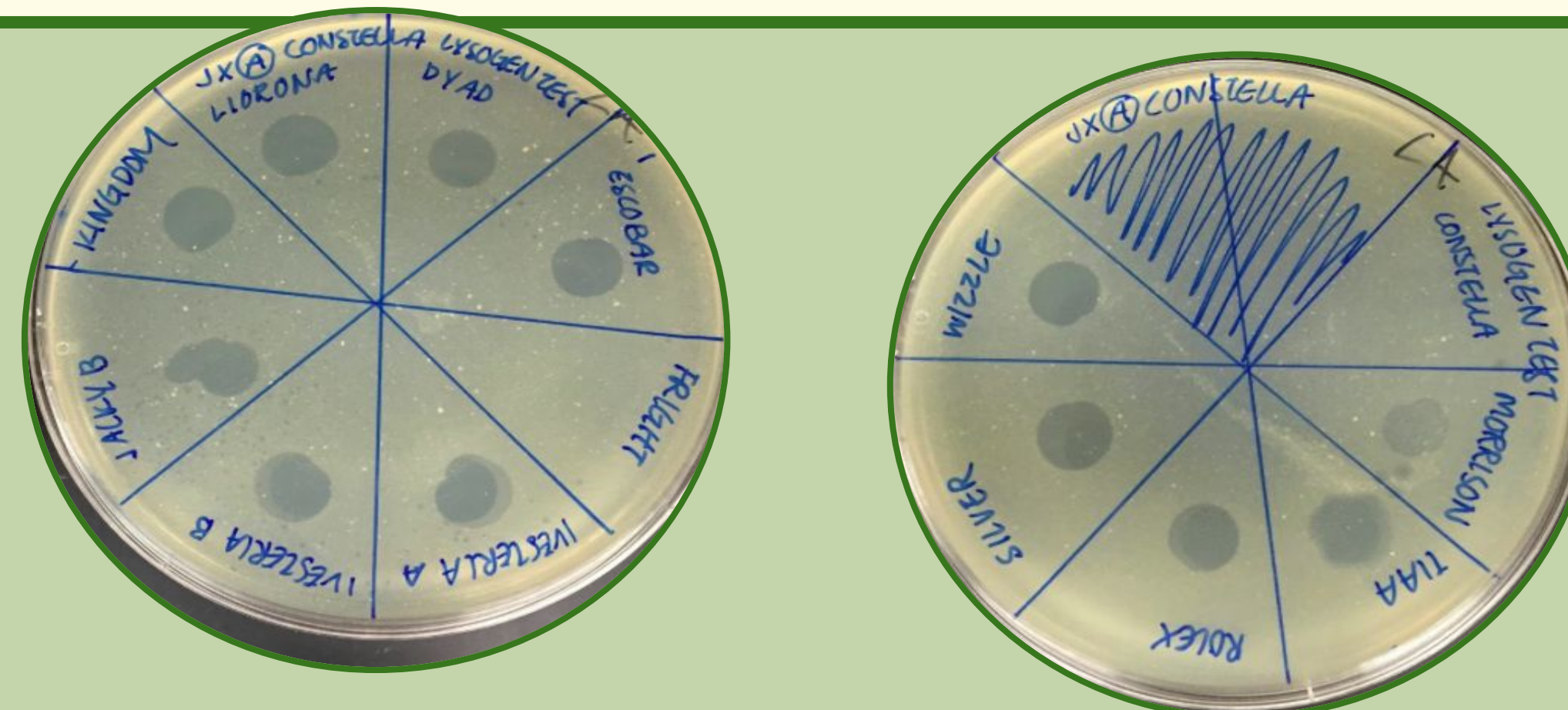
Acknowledgements:

Thank you to Julie Xu and all who helped isolate and run tests on Constella. Additionally, thank you to Dr. Alvey for his guidance and HHMI for funding the SEA-Phages program at Illinois Wesleyan University.

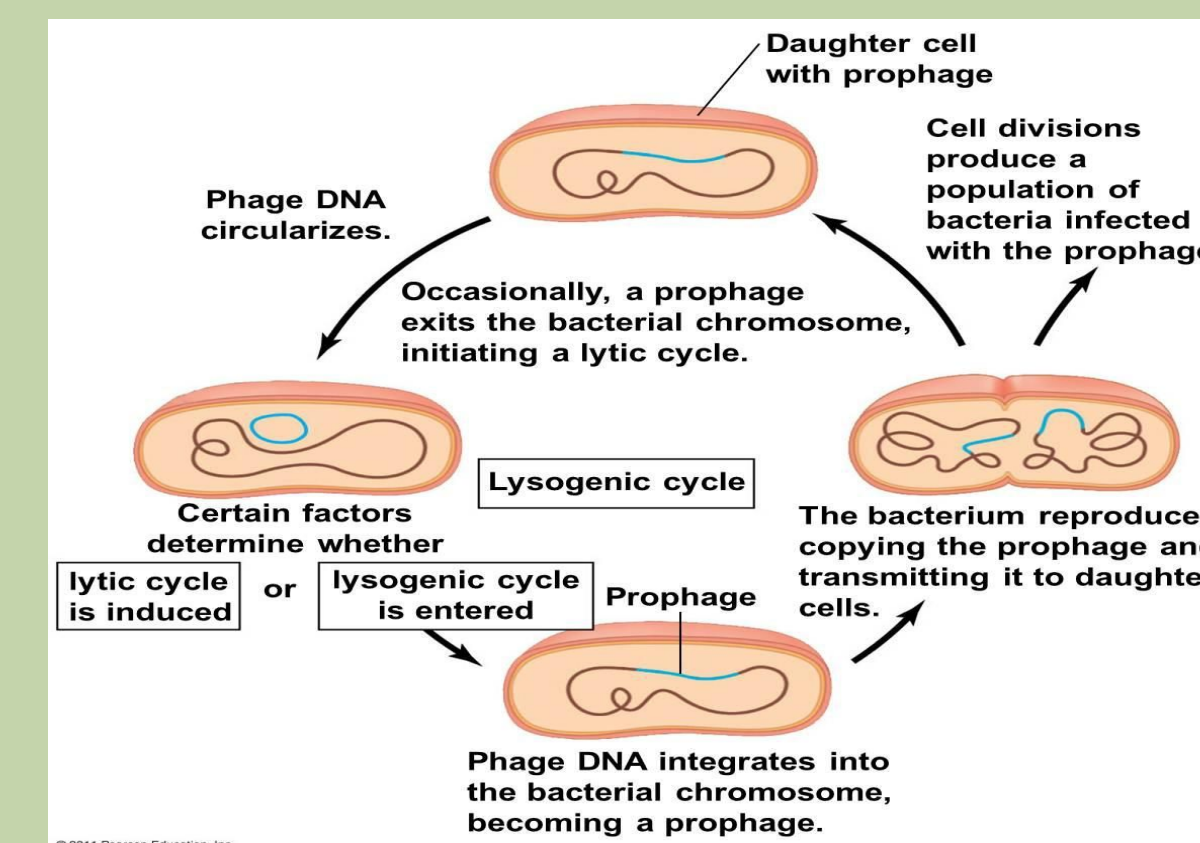
Immunity:

The following class phages formed plaques on the Constella lysogen:

- Escobar
- Doddsville
- Dyad
- JackyB
- Rolex

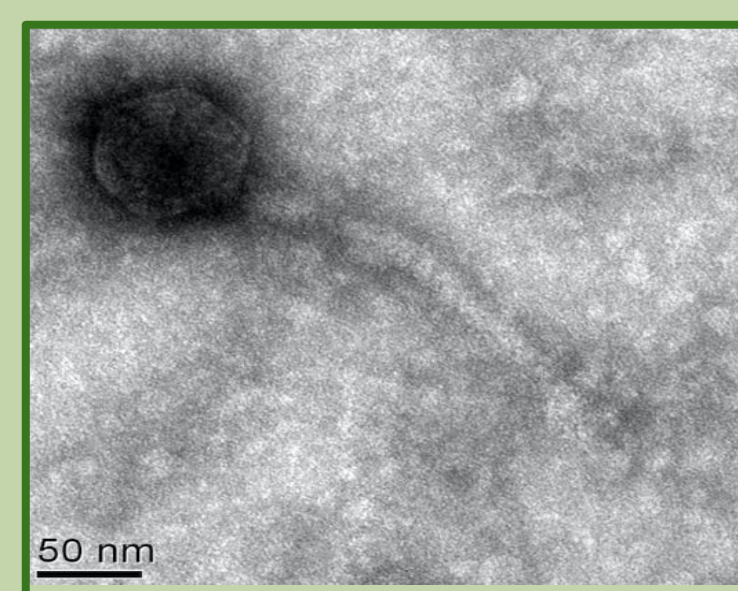


Plaque formation occurs when one phage that is unrelated to another phage forms a plaque on the lysogen of that unrelated phage. The plaque formation with the above class phages indicates that they are likely unrelated to Constella. The exception was Fright which did not form a plaque, an indication that Fright and Constella are relatives. As expected, the Constella phage did not form a plaque on its own lysogen.



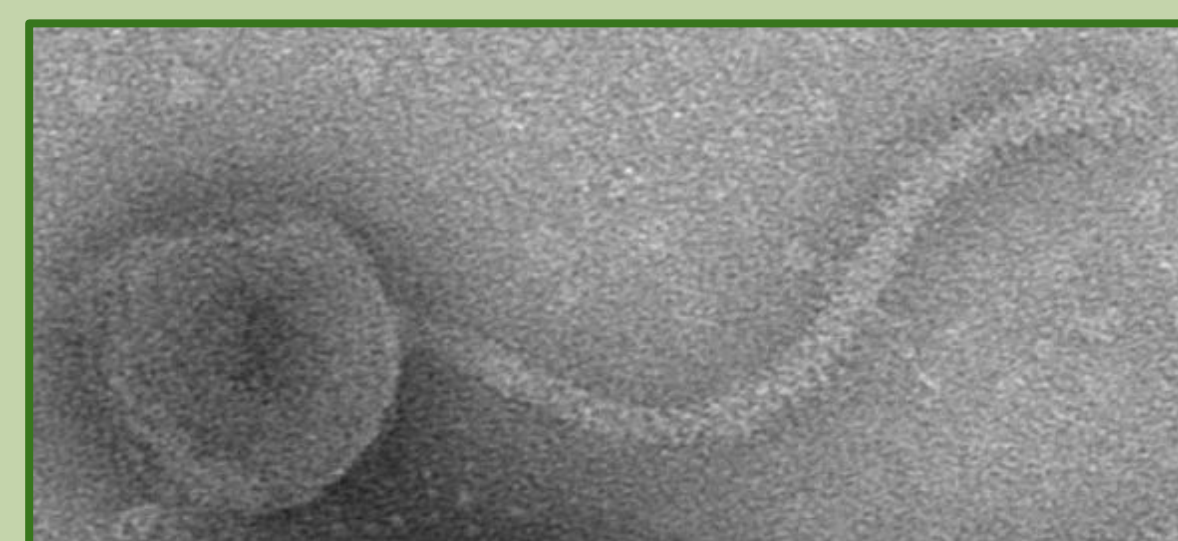
TEMs of J Phages:

Constella:



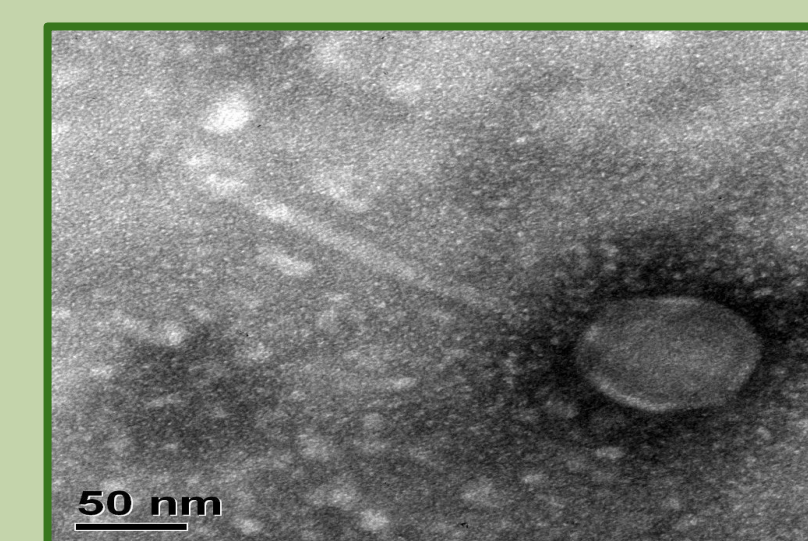
Capsid=77 nm, Tail=210 nm

DmpstrDiver:



Capsid=73 nm, Tail=217 nm

Squint:



Capsid=75 nm, Tail=225 nm

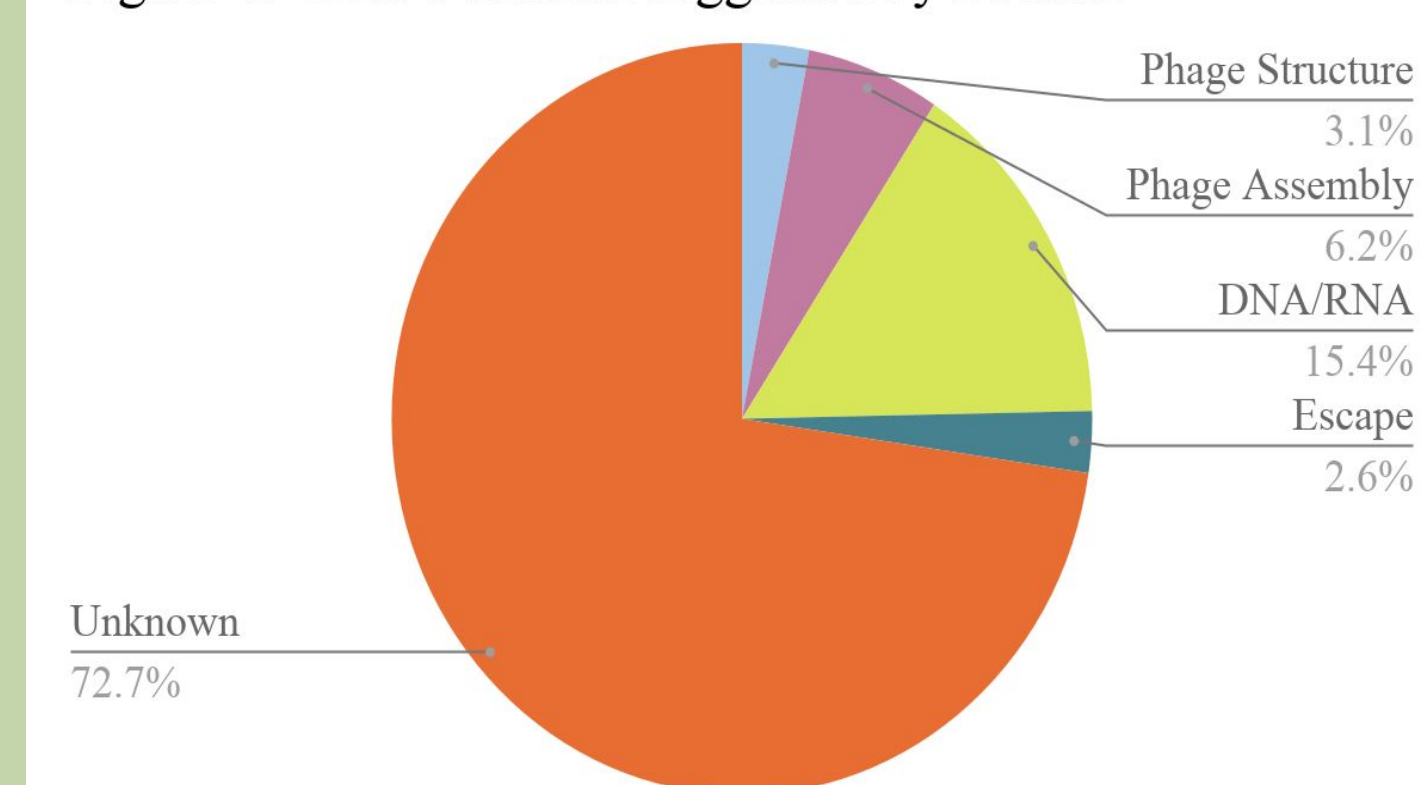
As expected, the J phages are siphoviridae, with long, flexible tails. They have similar tail lengths and even more similar capsid diameters. The similarity of capsid diameters suggests the phages hold about the same amount of genetic material.

Table 1: Phage Genome Comparisons

	# of Base Pairs	# of Genes	GC Content
Constella	110,169	235	60.80%
Squint	110,240	239	61.00%
DmpstrDiver	112,285	242	60.60%

Gene Annotations:

Figure 4: Gene Function suggested by Pecaan



Results:

Initial PCR and morphology TEM results suggested that Constella belonged to the F phage cluster. However, genomic sequencing revealed that Constella was actually a J phage, a rare phage that accounts for about 2% of the phages discovered thus far. Constella shares common characteristics with other J phages, including the ability to form a lysogen along with having an average base pair number of 110 kilo base pairs. Additionally, Constella also had similar capsid diameter and tail length measurements compared to other sequenced J phages.

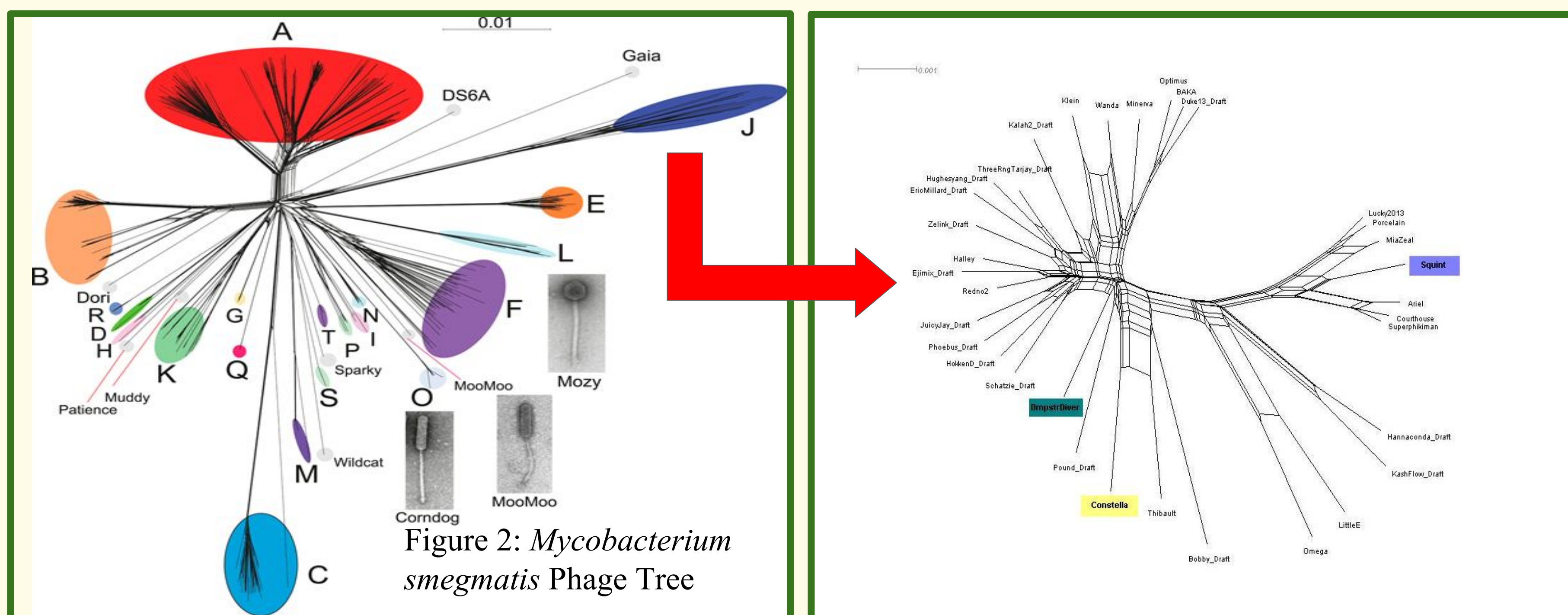


Figure 2: *Mycobacterium smegmatis* Phage Tree



Figure 5: Gene Map of DmpstrDiver, Constella, and Squint. Purple areas show 100% nucleotide identity