

INTRODUCTION:

In the past several years, bacteriophage research has accelerated. Despite these recent advances, only six bacteriophages that infect *Rhodobacter capsulatus* (*Rc*) strain YW1 were currently known. Through our efforts, six new phages that infect YW1 were discovered, and characterized using immunity and host-range testing. Dormio, McDreamy, and Tiptonus were also characterized using genomic data. McDreamy and Tiptonus were discovered in the Bloomington-Normal area, while Dormio was found in the Chicago area. Purified DNA of all three phages was sent to North Carolina State University to be sequenced. Sequence files were annotated using bioinformatic resources and compared to past data found for other *Rc* bacteriophages.

MATERIALS AND METHODS:

- Genomes of Dormio, McDreamy, and Tiptonus were annotated using DNA Master.
- ORFs, potential start sites, Shine-Delgarno scores, similar ORFs in other phages, and other features of the genome were identified
- Potential functions were assigned using HHpred, phagesdb.org, and NCBI BlastP.
- Phamerator was used to create diagrams comparing the relatedness of phages, indicating possible clusters.

Rc PHAGE CLUSTERS:

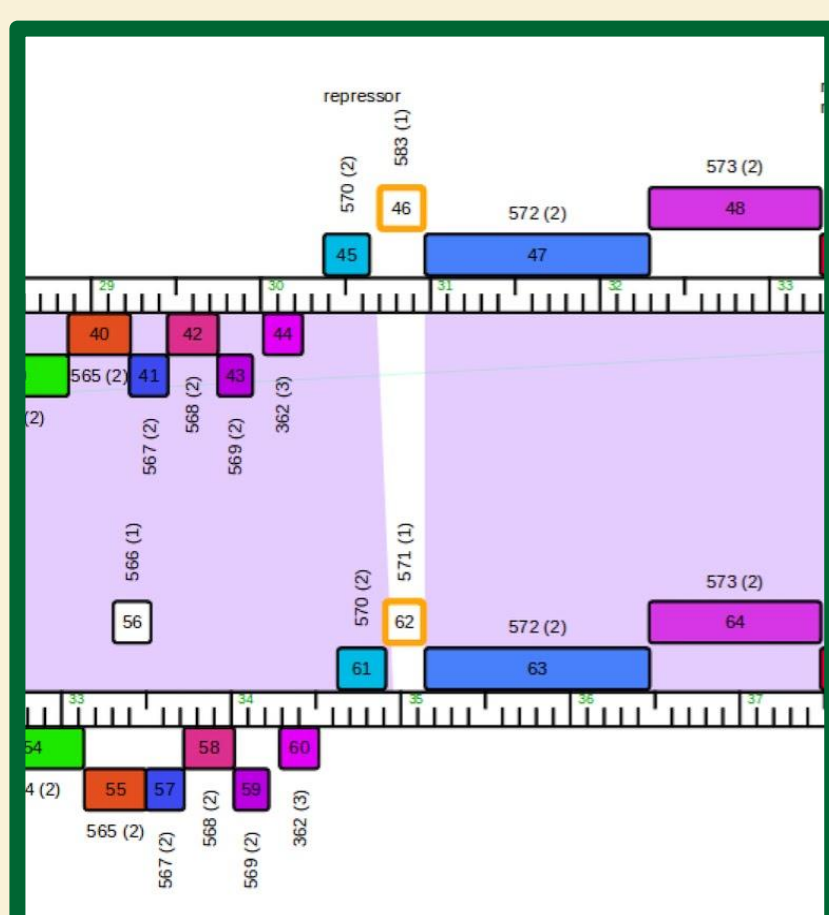
- Known *Rc* phages were grouped into three clusters (see Table 1) based on DNA similarity
- Cluster A: Average base pair (bp) length= 36,044.
- Cluster B: Average bp length= 44,345.
- Cluster C: Average bp length= 39,634.
- The phages that do not fit into any of the three clusters are Single Cluster phages (Singletons), with each phage representing its own unique cluster.

Table 1: Phage Clusters

Cluster	Members
Cluster A	Rhea, Cronus, Saxon
Cluster B	Titan, Spartan
Cluster C	Oceanus, Dormio
Singletons	RcapMu, RcapNL, RC1, McDreamy, Tiptonus

DORMIO RESULTS AND DISCUSSION:

- Dormio is very similar to Oceanus creating a new cluster (C) from a what was previously a singleton.
- A difference between Dormio and Oceanus near their repressor genes may account for why Dormio can infect the *Rc* B10 strain, and Oceanus cannot.

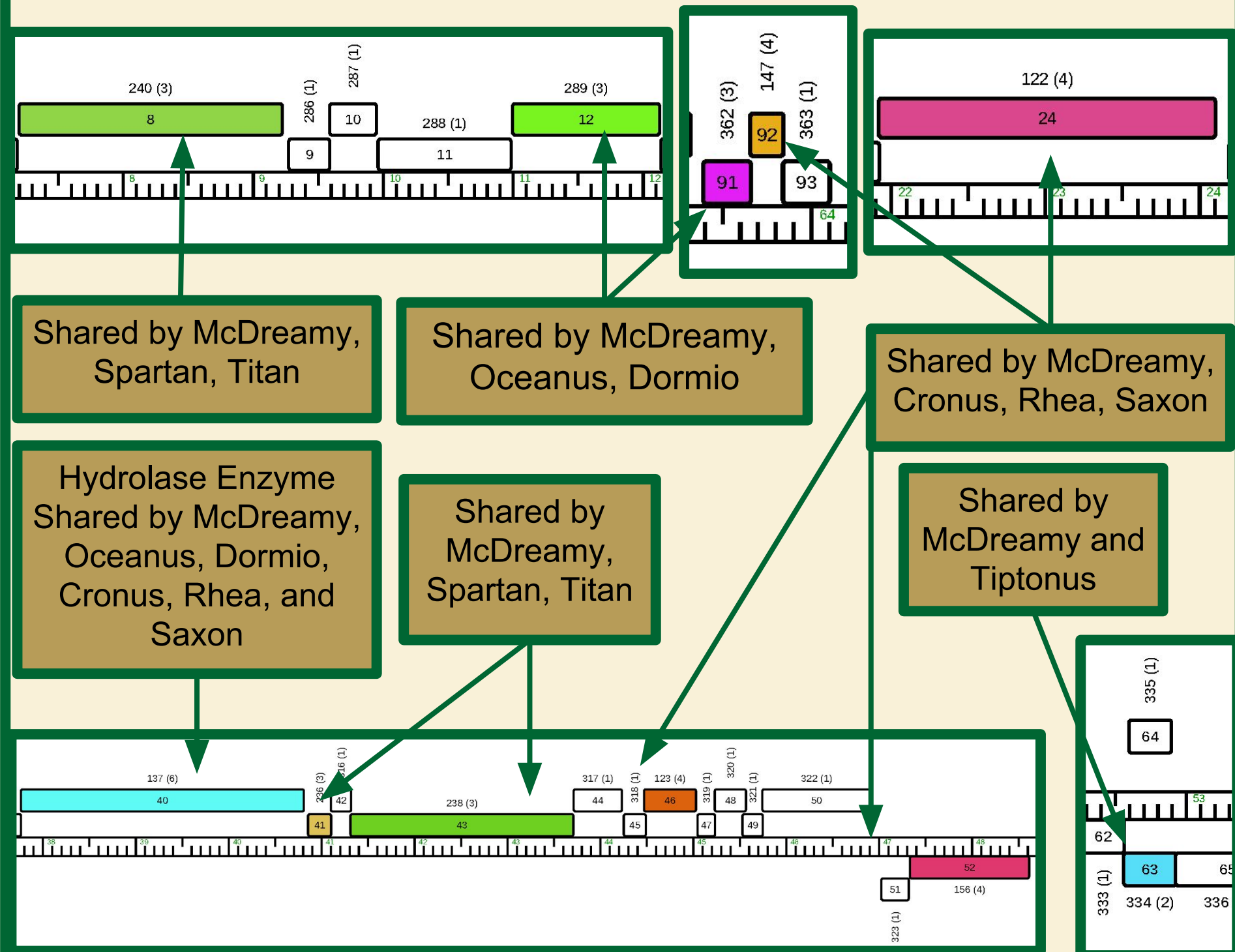


Left: Image A
 This is a map of the repressor and post-repressor genes of Dormio and Oceanus

Below: Image B
 Comparison map of Dormio and Oceanus.

MCDREAMY RESULTS AND DISCUSSION:

- Demonstrates mosaicism with all currently known clusters and the singleton Tiptonus.
- McDreamy, Dormio, Oceanus, Rhea, Saxon, and Cronus share a hydrolase enzyme, indicating that they likely have a shared "ancestor" phage.



TIPTONUS RESULTS AND DISCUSSION:

- Longest genome of all *Rc* phages currently known.
- Shares very few genes with other known *Rc* phages,
- A lysogen assay was done, and it was determined that there is a high likelihood that Bellator, and Titan are related to Tiptonus more so than originally thought.

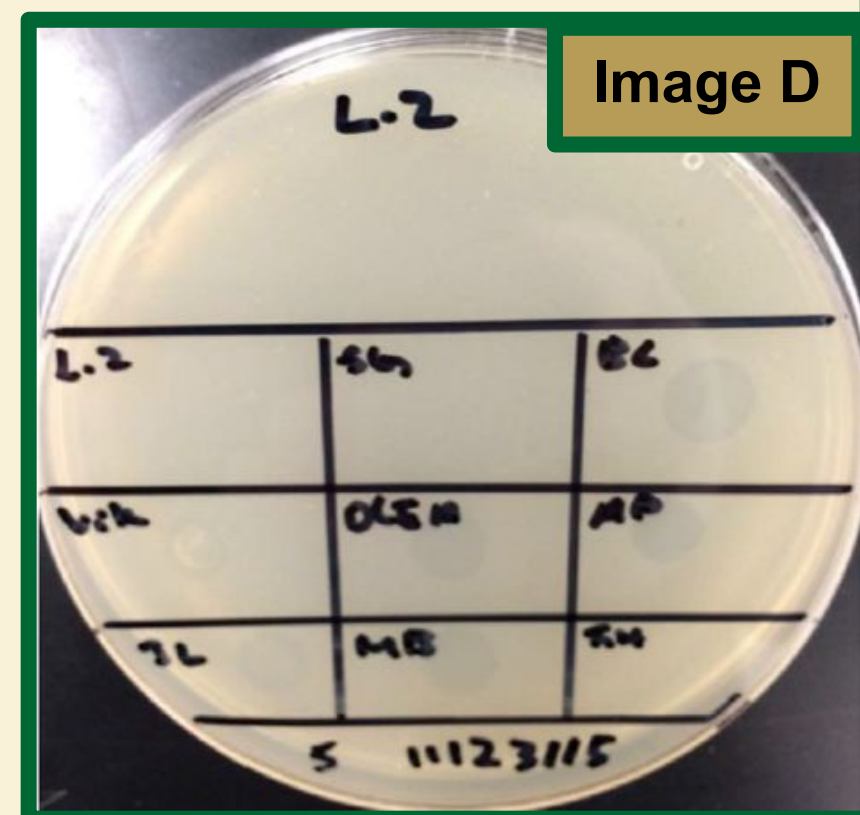
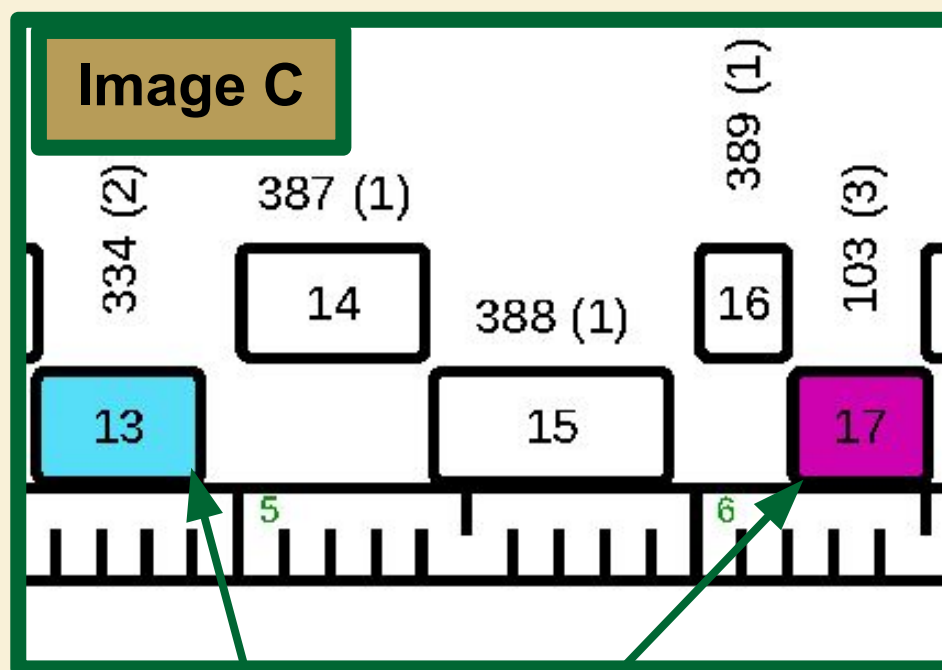


Image D Key: Ignore L.Z. at the very top. On grid:
 L.Z.—Tiptonus, SG—Bellator, BC—Dormio, Vik—Viking, Ocea—Oceanus, AP—McDreamy, JL—FrancisLouise, MB—DrBanner, Titan

Table 2: Comparison of Genomes

	Number of Genes	GC Content	Number of Basepairs
Average <i>Rc</i> Phage (before our work)	54.56	62.531%	39,072
Dormio	73	64.083%	41,656
McDreamy	104	59.594%	53,806
Tiptonus	148	53.806%	95,520
Average including new phages	67.92	62.068%	45,278
<i>Rc</i> YW1*	3481	66.6%	3,642,667

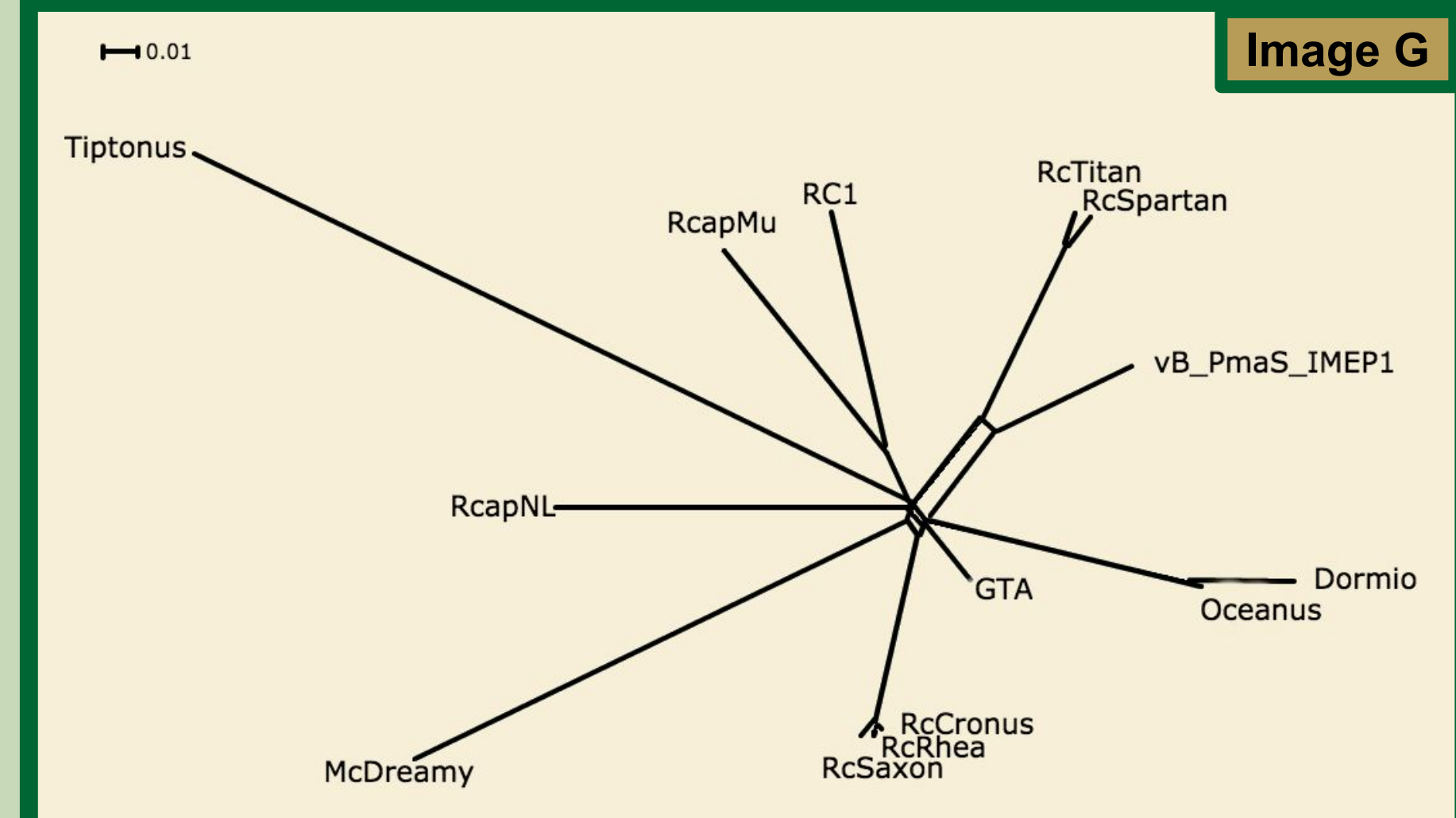
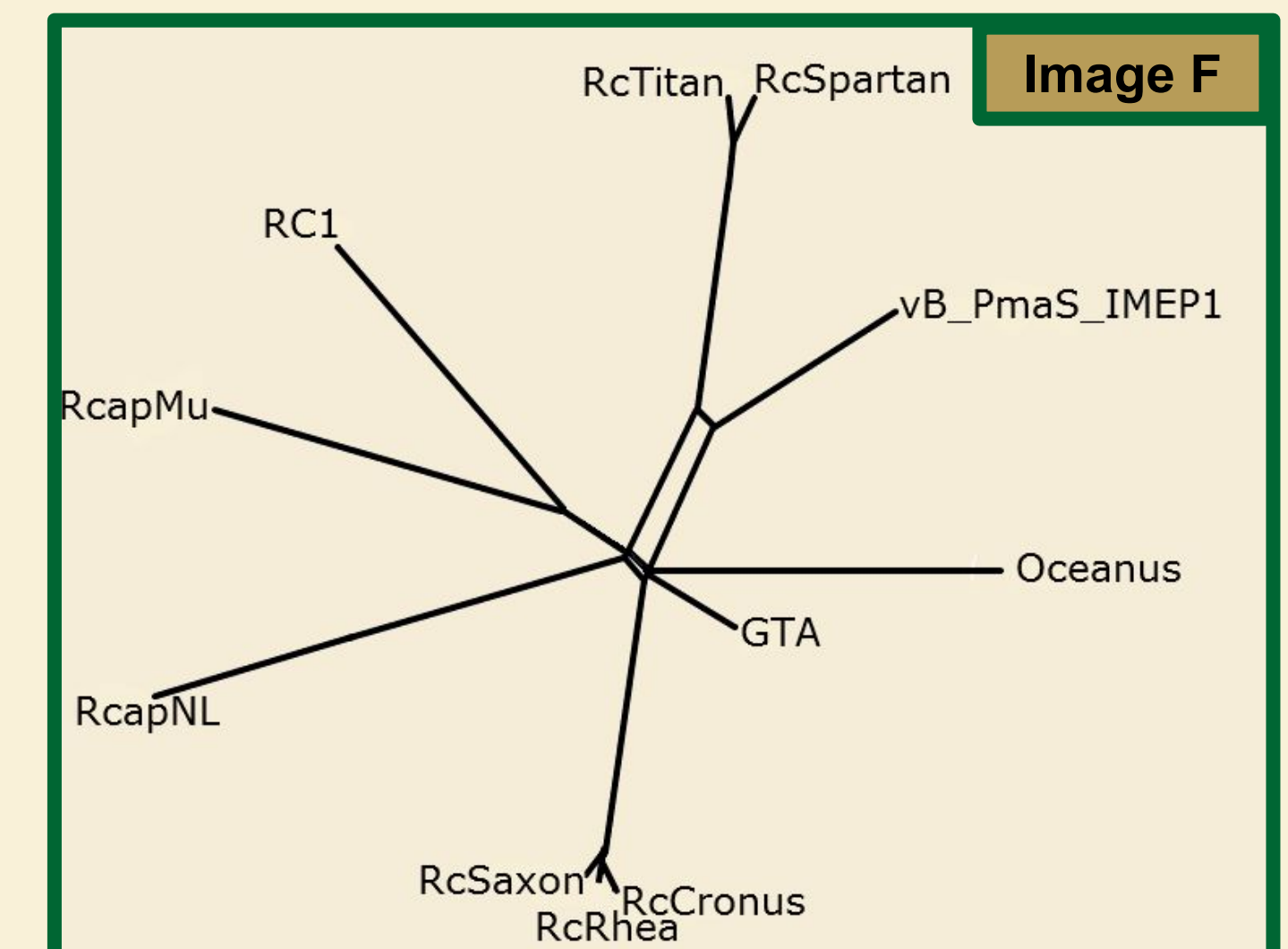
*Taken from Pathosystems Resource Integration Center

R.c phages "phylogenetic" comparison:

Image A (below) A Splitstree map of the inter-relatedness of the known *Rc* phages, as well as a *Paracoccus* phage (vB_PmaS_IMEP1), and GTA, a *Rc* phage remnant integrated in the *Rc* genome.

Image B (below) A Splitstree map incorporating our three newly found phages. Tiptonus and McDreamy are singletons representing new clusters, while Dormio groups with Oceanus in cluster C.

GenBank Ascension Numbers: RcCronus (KR935217), RcRhea (KR935216), RcSaxon (KT253150), RcSpartan (KR935215), RcTitan (KR935213), RC1 (AGH58045.1), RcapNL (AFK66511.1), RcapMu (AER29955.1), vB_PmaS_IMEP1 (AJD83159), GTA (AAF13179.1)



CONCLUSIONS:

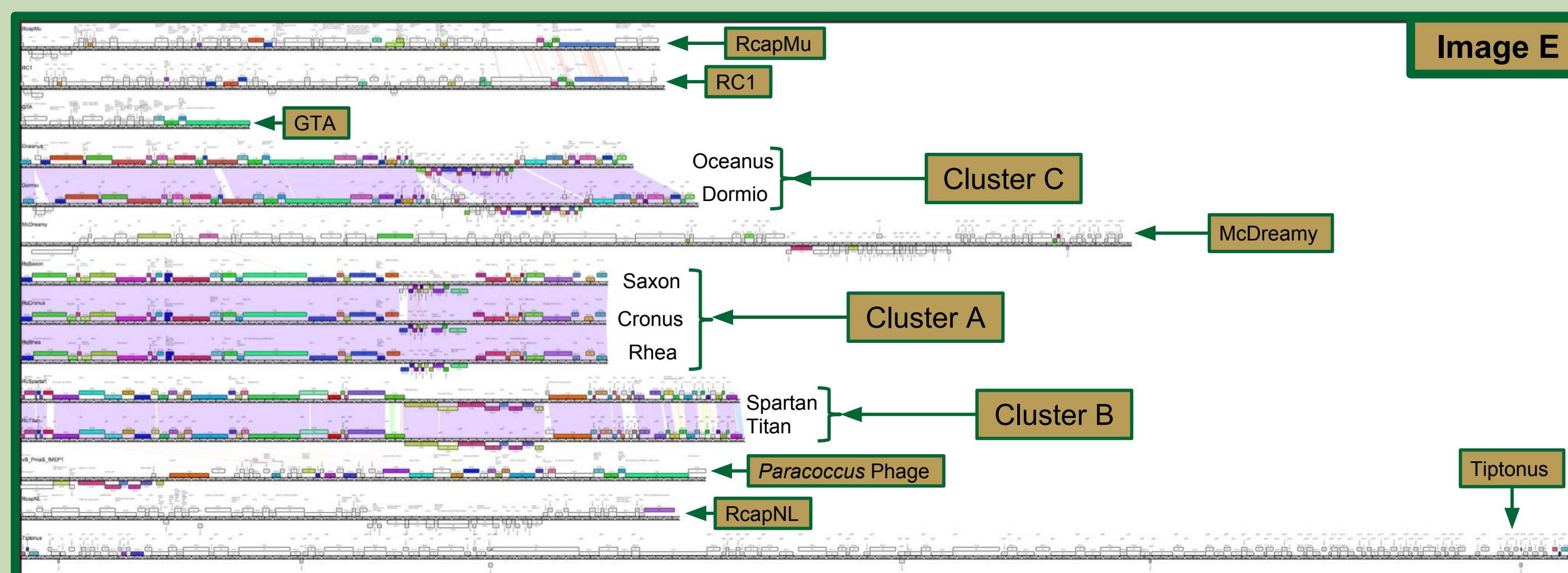
- Both McDreamy and Tiptonus have a genome length significantly greater than any previously discovered *Rc* phages, suggesting that they may represent two new *Rc* clusters.
- Dormio has helped create a new *Rc* phage cluster.

FUTURE DIRECTIONS:

- The three phages that were found and not sequenced could be sequenced, once purified DNA is obtained.
- A true lysogen test can be done to help determine whether or not the lysogen made by Tiptonus is a true lysogen.
- Upon a completion of genome annotation, the information will be sent to GenBank.
- Determine the effect of the orpham after the repressor gene in Dormio in its ability to infect the *Rc* strain B10.

REFERENCES/ACKNOWLEDGEMENTS:

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* Faculty Advisor