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SEA

PHAGES

9th annual symposium

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PROGRAM BOOK

Agenda

Abstracts

General Information

- Logistics Summary
- Shuttle Bus Schedule
- Venue and Hotel Addresses

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# SEA PHAGES

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AGENDA

# 9<sup>TH</sup> SEA PHAGES SYMPOSIUM

Friday, June 9, 2017

3:00 PM – 4:30 PM	CHECK-IN POSTER SET-UP STUDENT PRESENTER PRACTICE PERIOD	RECEPTION DESK, THEN GALLERY LOBBY AUDITORIUM
4:30 PM - 6:00 PM	STUDENT DEBRIEFING	AUDITORIUM
5:15 PM - 6:15 PM	FACULTY MEETING	SEMINAR ROOM COHORT 10 IN SYNAPSE SWIFT TEAM IN SYNAPSE
6:00 PM - 7:15PM	DINNER	DINING ROOM
7:15 PM - 7:30 PM	WELCOME AND REMARKS	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
7:30 PM - 8:45 PM	KEYNOTE I Dr. Rachel Dutton University of California-San Diego	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
8:45 PM - 9:30 PM	CHEESE SOCIAL	LOBBY
9:00 PM – 11:00 PM	HOTEL SHUTTLES – EVERY 30 MINS <i>Hotel guests will be shuttled to their hotels. Shuttles will leave for the hotels every half hour starting at 9:00 PM. The last hotel shuttles run at 11:00 PM.</i>	DRIVEWAY LOOP ENTRANCE

# 9<sup>TH</sup> SEA PHAGES SYMPOSIUM

Saturday, June 10, 2017

7:00 AM - 8:30 AM	BREAKFAST	DINING ROOM
<i>Hotel guests will be shuttled from their hotels to Janelia Research Campus starting at 6:45 AM. Shuttles run every 15 minutes. The last shuttles run at 8:00 AM.</i>		
8:30 AM - 8:45 AM	INTRODUCTORY REMARKS	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
8:45 AM - 10:00 AM	KEYNOTE II Dr. Graham Hatfull University of Pittsburgh	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
10:00 AM - 10:15 AM	BREAK	LOBBY
10:15 AM - 12:00 PM	POSTER SESSION I   ODD-NUMBERED POSTERS	LOBBY
12:00 PM - 12:30 PM	GROUP PHOTO	DRIVEWAY LOOP ENTRANCE
12:30 PM - 2:00 PM	LUNCH	DINING ROOM
2:00 PM - 3:30 PM	STUDENT ORAL PRESENTATIONS I MODERATOR: MIRIAM SEGURA-TOTTEN	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM

**From the Dirt to the Dish: 12 new *Gordonia* phages.**  
*Elizabeth Koning, Jennifer Van Dalen - Calvin College*

**Discovery and characterization of bacteriophage LuckyBarnes.**  
*Savannah Underwood, Logan Ryals - University of Southern Mississippi*

**The Isolation and Annotation of Prolate-Head Bacteriophage Satis and Three Other Highly Novel *Streptomyces* Bacteriophage.**  
*Kelly Hartigan, Nicole Curnutt - Washington University in St. Louis*

**Isolation and Characterization of Five *Gordonia* Bacteriophages from Tropical Environments of Puerto Rico.**  
*Monica C. Correa, Raelys DeLeon-Fernandez - University of Puerto Rico at Cayey*

**King Solomon's tale – exploring ways to increase phage-hunting success and find them homes.**  
*Amara Ejikemeuwa, Brett Farran - University of West Florida*

**Host range project: Investigating if phage isolated from *Mycobacterium smegmatis* can infect pathogens of fish, amphibians and humans.**  
*Delilah Hahn, Ryan Kilduffh - Old Dominion University*

# 9<sup>TH</sup> SEA PHAGES SYMPOSIUM

Saturday, June 10, 2017 (continued)

3:30 PM - 3:45 PM	BREAK	LOBBY
3:45 PM - 5:30 PM	POSTER SESSION II   EVEN-NUMBERED POSTERS	LOBBY
5:30 PM - 7:00 PM	DINNER	DINING ROOM
7:00 PM - 8:30 PM	STUDENT ORAL PRESENTATIONS II MODERATOR: EVAN MERKHOFFER	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM

Isolation and Gene Analysis of North Dakota Phage Old Ben, Maryland Phage Lulumae, and Experiments with Alternative Bacterial Hosts

*Luke Keller, Grace Burns - University of Mary*

Phinding Genomic Gems Using DOGEMS

*Fariyah Miah, Victoria Pobok - University of the Sciences in Philadelphia*

Exploring New Terrae-tories with Gordonia Phages

*Maridaria Lillis, Mariana Morales - Nyack College*

Actinobacteriophage holin protein identification and classification using protein motifs, structural analysis, and comparative bioinformatics

*Terran Gilbreath - Washington State University*

The Diversity of Phages Compared According to their Hosts

*Emma Eisemann - James Madison University*

Preliminary Cluster Typing of 38 Mycobacteriophages Discovered at Western Carolina University and Surprisingly High Sequence Identity of a Rare Cluster M Bacteriophage

*Dylan Rood, Henry Salvo - Western Carolina University*

8:30 PM - 9:30 PM	SOCIAL	LOBBY
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9:00 PM – 11:00 PM	HOTEL SHUTTLES – EVERY 30 MINS	DRIVEWAY LOOP ENTRANCE
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*Hotel guests will be shuttled to their hotels. Shuttles will leave for the hotels every half hour starting at 9:00 PM. The last hotel shuttles run at 11:00 PM.*

# 9<sup>TH</sup> SEA PHAGES SYMPOSIUM

Sunday, June 11, 2017

7:00 AM - 7:30 AM CHECK-OUT

*Hotel guests will be shuttled from their hotels. Shuttles will leave for Janelia Research Campus starting at 6:45 AM. The last shuttles run at 8:00 AM. All guests must check out of their rooms before breakfast. Luggage may be stored in the Gallery and Synapse Meeting Room.*

7:00 AM - 8:30 AM BREAKFAST

DINING ROOM

8:30 AM - 10:00 AM STUDENT ORAL PRESENTATIONS III

STUDENTS IN AUDITORIUM

MODERATOR:

FACULTY IN SEMINAR ROOM

CARLEITTA PAIGE-ANDERSON

**Comparative Analysis of Mycobacteriophage Minor Tail Protein Carbohydrate Binding Module Polymorphism and Host Range.**

*Connor DeJager, Megan Perez - LeTourneau University*

**B1 is for is for Bacteriophage.**

*Pauline Good, Andrew Jurgielewicz - Drexel University*

**The Highs and Lows of Phage Diversity: introducing Cuke, Flapper and SallySpecial, a singleton mycobacteriophage and two novel Gordonia phage.**

*Alyssa Jones, Katherine Larochelle - University of Maine, Honors College*

**Investigating Four Gordonia Bacteriophages: Apricot and the Three CR.**

*Gunnar Goetz, Sarah Schmidt - Carthage College*

**Analysis of Putative Repressor/Antirepressor Induction System in Cluster P Mycobacteriophages Arib1 and Brusacoram.**

*Amanda Rieffer, Melanie Reese - College of St. Scholastica*

**A host of phages: Diversity in morphology, genomes, and host specificity exhibited by phages isolated in three Actinobacteria hosts.**

*John Lowery, Caleb Rossin - University of Wisconsin-River Falls*

10:00 AM - 10:30 AM BREAK

LOBBY

# 9<sup>TH</sup> SEA PHAGES SYMPOSIUM

Sunday, June 11, 2017 (continued)

10:30 AM – 11:15      FACULTY PRESENTATIONS      STUDENTS IN AUDITORIUM  
MODERATOR:      FACULTY IN SEMINAR ROOM  
VICTORIA FROST

Potential of Mycobacteriophage as Candidates for Phage Therapy - Identification of Phage that Infect Nontuberculosis Mycobacteria Pathogens

*Ann Findley - University of Louisiana at Monroe*

A Genome in a Day; Lessons from the Updawg Genome Annotation Hackathon

*Erin Doyle - Doane University*

Identification of a Role for Mycobacteriophage Butters-encoded Proteins in a Host Defense Mechanism Against Viral Attack

*Vassie Ware - Lehigh University*

11.15 AM – 11.45 AM      EXPLORATION OF PHAGE SENSITIVITY & RESISTANCE      STUDENTS IN AUDITORIUM  
FACULTY IN SEMINAR ROOM

11:45 AM - 12:00 PM      CLOSING REMARKS      STUDENTS IN AUDITORIUM  
FACULTY IN SEMINAR ROOM

12:00 PM - 1:30 PM      LUNCH      DINING ROOM

12:00 PM - 1:30 PM      POSTER REMOVAL AND DEPARTURE      LOBBY  
*All guests will be shuttled to their destination. Shuttles will leave for the airports at 1:00 PM.  
The shuttle to HHMI Headquarters for the Genome Annotation Workshop departs at 2:00 PM.*



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# SEA PHAGES

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ABSTRACTS

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #001

Albion College  
Albion MI

Corresponding Faculty Member: Brad Cavinder (bcavinder@albion.edu)



Brielle Brown



Elise Anderson

### ***Isolation, Characterization and Genome Annotation of Two Siphoviridae Group B Cluster Members from the Albion College Campus***

Brielle Brown, Elise Anderson, Brad Cavinder, Ola Olapade

Bacteriophages, commonly known as phages, are viruses that infect bacteria, requiring a bacterial host in order to replicate their genomes and produce new virions. Due to the abundance of phages and their ability to kill bacteria, research of phage therapy, using phages for the treatment of infections by pathogenic bacteria, is ongoing. The purpose of this HHMI SEA-PHAGES sponsored project was to isolate and purify phages that infect *Mycobacterium smegmatis*, and then to extract, sequence, and annotate the genomes of two isolates. Environmental samples were collected from various sites in Albion, Michigan. Phage enrichment was performed before spot testing on *M. smegmatis*. Phages from spot test plaques were serially diluted to isolate individual phage which were grown to high titer for genomic DNA extraction. The DNA was sequenced through the SEA-PHAGES project. Our phages Tincidunsolum and Brownie5 were found to be members of the Siphoviridae family of double-stranded DNA viruses and are both in the B2 subcluster. The annotation portion of the project utilized a variety of bioinformatics tools, including DNA Master, Phamerator, Starterator, NCBI Blast, and HHPred, to evaluate the accuracy of automated gene annotations called by GeneMark and Glimmer. Modifications to the annotations included the deletion of erroneously called genes, a programmed frameshift, and adjustment of probable start sites. We used Aragorn and tRNA ScanSE 2.0 to check for tRNA and tmRNA genes, and none were found. These findings will aid in our understanding of phage biology, contributing in a broad sense to the development of phage therapy.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #002

Austin Community College  
Austin TX

Corresponding Faculty Member: Ana Maria Valle-Rivera (anamaria.valle-rivera@austincc.edu)



Micasia Cash



Cassandra L Woliver

### ***Isolation and Genome Annotation of mycobacteriophage "ArcusAngelus", a Siphoviridae Group F Cluster F1 from Austin Community College***

Micasia Cash, Cassandra L Woliver, Kevin Britt, Michelle Vang, Patricia Bal, Kennedy M Blanton, Tyler Busk, Tram Dang, Arianne E Dillard, Austin B Eickhoff, Jennifer Gallardo, Carissa L Gragg, Stephanie D Gunkel, Ara M Hunt, Jessica M Kohanek, Bryce C LaChance, Kristine M Laqui, Nihitha Nukala, Mary K O'Malley, Zarae R Pena, Francisca Porras, Christopher G Riggs, Yu Syuan T Yeh, Martha Aguilera, Luis Argaez-Licea, Tara Belles-Elsea, Christina Bennett, Daisy Gutierrez, Luke Lindsay, Davalynn Quiroz, Joshua Rollins, Ana Maria Valle-Rivera

It was the first year for submissions of mycobacteriophages to the SEA PHAGE program from the Central Texas area. We were able to discover a novel phage 'ArcusAngelus' isolated from the host bacteria *Mycobacterium smegmatis*, mc2155. The isolation method was enrichment. The plaques produced were small and clear. Electron microscopy was performed by the facility at the University of North Dallas. The electron micrographs showed that the virus morphology was consistent with the Siphoviridae. The genome of 'ArcusAngelus' was sequenced by the Pittsburgh Bacteriophage Institute using Illumina Sequencing. The genome was annotated using bioinformatics software including DNA Master, NCBI's Blastp, Phamerator and Starterator. The genome had 113 predicted genes. We found protein functions that match functions for bacteriophages in the national database as well as unknown function proteins by comparing the phage 'ArcusAngelus' with other phages isolated from the same host.

## 9th Annual SEA-PHAGES Symposium Abstract

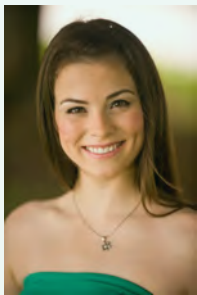
Poster #003

Baylor University  
Waco TX

Corresponding Faculty Member: Tammy Adair (tamarah\_adair@baylor.edu)



Joshua Baker



Christian Sessa

### ***BEARS in the SEA Unearth *Arthrobacter* Phage Diversity Along Waco's Brazos River***

Joshua Baker, Christian Sessa, Caroline Addison, Niru Ancha, Thomas Eckenrode, Haley Everroad, Christina Gaw, Jake Hanna, Julia Hawes, Cori Hughes, Alec Ingros, Roshni Jaffery, Emily Johnson, Pranav Kapoor, Navya Katragadda, Niharika Koda, Stu Mair, Alex Munoz, Micheal Munson, Noah Patton, Madison Powell, Andrea Springman, Natalie Widdows, Daniel Zeter, Lathan Lucas, Ashley Young, Jennifer Wilson, Tamarah Adair

Bacteriophage are prolific throughout our biosphere, yet little is known about their genome. *Arthrobacter* phage infect *Arthrobacter*, a genus of common soil bacteria known to metabolize a variety of organic substrates. An increased interest in *Arthrobacter* phage has resulted in over 150 sequenced genomes since 2012. These phages were all isolated on *Arthrobacter* sp. 21022 and are classified in 12 clusters based on nucleotide similarity. As this area of phage genomics grows, a more efficient method of clustering phages that does not require whole genome sequencing would facilitate further studies in this field. Our research adds the annotations of four *Arthrobacter* phage genomes from four different clusters to the phagesdb.org database: Lore (AN), Shrooms (AL), Caterpillar (AU), and Nubia (AK) and proposes a new method for cluster identification using phage lysates. Phages were isolated from soil in various locations in Waco, TX, and purified on host *Arthrobacter* sp. 21022. DNA was purified and sequenced using Illumina Sequencing and annotated using DNAMaster. Genes and their putative functions were predicted using Glimmer, Genemark, NCBI BLASTp, and

HHpred. Furthermore, comparative analysis using Phamerator and Starterator supported gene annotations. Overall annotations reveal that *Arthrobacter* phage Lore is similar to the other ~15,550 base pair phages of the AN cluster. Interestingly a function not previously called in AL phages was discovered for Shrooms gene 51 through an HHpred hit that matched a MazG nucleotide pyrophosphohydrolase. Caterpillar was unique in that its genome contained four large intergenic gaps. Nubia, like other AK phages, contained a frameshift within the tail assembly chaperone gene. Promoter and terminase analysis was conducted in each cluster using DNAMaster, BPROM, and ARNold. Sigma 70 like promoters were identified in the gaps between the forward and the reverse genes of Lore, Nubia, and Shrooms. Probable rho-independent transcription terminase sequences were also identified in each of the four phages. Tape measure protein (TMP) sequences from each cluster were compared using Gepard dotplots and Mega7 was used to produce a phylogenetic tree. The results indicate that TMP single-gene clustering is identical to whole genome clustering. Therefore, we used the variable regions of the TMP genes from each cluster to design 12 unique primer sets. In the future, these primers will be tested against a panel of known *Arthrobacter* phage. *Arthrobacter* phage diversity is predicted to be high, similar to Mycobacteriophage diversity, based on the large number of clusters already recognized. As in Mycobacteriophage research, TMP analysis has the potential to provide an efficient way to cluster *Arthrobacter* phage in the laboratory without sequencing the entire genome.

# SEA PHAGES

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## 9th Annual SEA-PHAGES Symposium Abstract

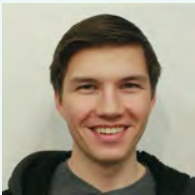
Poster #004

Brigham Young University  
Provo UT

Corresponding Faculty Member: Julianne Grose (julianne\_grose@byu.edu)



**Brittany A Colby**



**Timothy S Ballard**

### ***The Bees and the Trees: 2016-2017 Phage Hunting at BYU***

**Brittany A Colby, Timothy S Ballard, Christopher P Farjardo, Jared L Krugar, Steven Duncan, Charles J Webb, Ruchria Sharma, Donald Breakwell, Sandra Hope, Julianne Grose**

American foulbrood and Fire blight are dangerous diseases that destroy beehives and fruit orchards across the United States respectively. The bacteria that cause these diseases are *Paenibacillus larvae* and *Erwinia amylovora* respectively, and the bacteriophages that infect these bacteria have been shown to combat the deadly pathogens. This year two sections of Phage Hunters with 22 and 18 students isolated a total of 45 phages. Twenty-four *Erwinia* phages and 21 *Paenibacillus* phages were isolated, and DNA was successfully isolated and sequenced from 24 of these phages. Of the 16 fully sequenced *Erwinia* phages, 11 were similar to previously sequenced *Erwinia* phages, while five made up one novel cluster and two singletons. Out of the 21 *Paenibacillus larvae* phages we discovered, our phage hunter team was able to sequence eight genomes. When we put our eight phages in a dot plot we found two major clusters with very high similarities. Four of them we were able to fully annotate and find that they were related to the Fern cluster. Throughout the process of phage isolation and annotation, our course adapted to many challenges and demands required of novel research. Altogether, our research is helping us to understand the evolution of the bacterial pathogens *Erwinia* and *Paenibacillus*, as well as to develop the field of phage therapy as an alternative cure for these destructive diseases.

Brigham Young University  
Provo UT

Corresponding Faculty Member: Julianne Grose (julianne\_grose@byu.edu)

## ***Weekly Exercises Aimed at Improved Understanding of Key Concepts for the Phage Hunters Classroom***

Steven Duncan, Christopher Fajardo, Julianne H Grose

Writing has been shown to increase basic understanding in the classroom and is critical to most scientific study. We have developed twenty-one exercises designed to increase understanding of key phage hunters concepts through enhanced writing. Twelve of these assignments are for use in the in situ section of the course, and cover a range of topics including sources of contamination, phage lifecycles, performing serial dilutions and calculating PFU, reading scientific articles, electron microscopy, phage DNA isolation, restriction enzymes, archiving phage, and reading scientific blogs. The final assignment is a blog in which students summarize their in situ experiences. Nine assignments are designed for the in silico portion of the class. They focus on genomic analysis concepts including phage packaging mechanisms, coding potential, dot plot alignment, identifying motifs in phage DNA and phamerator as well as starterator. The final assignment is a group abstract students will use to prepare their final poster for the course. All twenty-one of the assignments are given two grades, one for accuracy (based on whether the answer is correct and comprehensive) and one for writing (based on whether the answer is clear and concise). Although no concrete analysis of these exercises has been performed, student-reported classroom experience has improved. We present these exercises in hope that they will be useful as tools to increase understanding in other phage hunters classrooms.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #006

Bucknell University  
Lewisburg PA

Corresponding Faculty Member: Marie Pizzorno (pizzorno@bucknell.edu)



Francesco P Satriale



Agustina Whamond

### ***The Mystery of the Disappearing Arthrobacterphage and the Analysis of Chipper1996 and Zorro, New Members of the AR and AK Clusters***

Francesco P Satriale, Agustina Whamond, Charles J Basralian, Katherine M Edwards, Matthew J Elander, Erin R Goldberg, Lauren M Gross, Mackenzie L Haney, Sophie Kong, Zachary J Kozick, Kenneth J Lopez, Nicole M Nisivoccia, Gabrielle Petruso, Chad R Reese, Jasmine Q Spinelli, Marie C Pizzorno, Emily L Stowe

During the fall of 2016 the Bucknell University Phage Hunters were happily isolating and purifying bacteriophage using *Arthrobacter* sp (ATCC 21022) as the host bacterium when all of a sudden the bacterial cultures became resistant to infection with any of the phage that had been identified. After trying various media formulations, growth temperatures and growth conditions to no avail, a new *Arthrobacter* sample was purchased from ATCC. Even the new cells appeared to be resistant to infection with all of the phage that had been isolated except Chipper1996 and Cheddar. Genomic DNA was purified from these two phage, along with Zorro which had been isolated in fall 2015, and submitted for sequencing. Genome sequences of Chipper1996 and Cheddar were identical and presumably represent contamination of phage between lab groups. Chipper is in the *Myoviridae* family, AR cluster and has a genome of 70090 bp. It is one base shorter than Tophat, which was isolated in summer 2015 at Bucknell. This one base deletion causes a frameshift mutation and results in gene 60 being split into two open reading frames, suggesting the protein product of this gene is not essential for lytic infection. Chipper encodes 112 genes with one block of five reverse genes. Several of these genes encode proteins with putative DNA modification functions, such as methylases and endonucleases. Zorro is in the *Siphoviridae* family, AK cluster and has a genome of 43562 bp. It has 7 base differences when compared to



Dino, also isolated at Bucknell in fall 2015. These 7 differences, which are mostly purine transitions, change four amino acids in four separate genes. The less conservative changes are in genes near the end of the genome and are likely to code for non-essential functions. While the 43 genomes in the AK cluster are relatively similar to each other, 25 contain three genes at the 5' end not found in the remaining 18 genomes. These 25 genomes also contain a different minor tail gene than the other phage. Zorro encodes 62 putative genes with a single block of 5 reverse genes. There are several interesting genes including gp49 that codes for a putative adenylosuccinate synthetase enzyme, which is involved in nucleotide metabolism. This gene is conserved in all of the AK genomes and in the genome of Ghobes, a singleton isolated from *Gordonia*. In addition, there is a block of genes (gp46-49) that encode possible zinc binding proteins. MEME identified several DNA motifs in both genomes that may be associated with transcriptional and/or translation start sites in the genomes of these phage. Further analysis of these repeats may determine if they are significant regulatory sequences for either of these phage.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #007

Calvin College  
Grand Rapids MI

Corresponding Faculty Member: Randy DeJong (rdejong@calvin.edu)



Elizabeth R Koning



Jennifer H Van Dalen

### ***From the Dirt to the Dish: 12 new *Gordonia* phages***

Katherine M DeHeer, Olivia S Harlow, Eric M Hayden, Allison D Karl, Evan A Klein, Katherine M Kim, **Elizabeth R Koning**, Emily G Kuburski, Olivia N Lamberg, Samuel P Lenz, Praise F Olatunde, Meghan A Schilthuis, Taylor N Smith, Kyle B Timmer, **Jennifer H Van Dalen**, Alexandra M VanDeWege, Matthew J Schepers, Mary R Horner-Richardson, John T Wertz, Randall J DeJong

Seventeen *Gordonia* phages with siphoviridae morphology were isolated from soils in Grand Rapids, Michigan, USA, using *Gordonia terrae* 3612. This result is a dramatic improvement over last year when we isolated and sequenced only one *Gordonia* phage in our first year using this host. Even so, two class members did not isolate a *Gordonia* phage after several attempts and therefore isolated three enterobacteriophages from soil using bacterial isolate *Citrobacter* sp. D22, of which one phage was sequenced, the myoviridae Thanatos. Thirteen of the *Gordonia* phages were sequenced, yielding additions to 5 clusters that had relatively few prior members: DC (4 previous members, we added Barb, Evamon, Fireball, Fugax, Portcullis, Valary, VanDeWege), DD (7, Lamberg), DE (10, Dexdert), DI (3, Bock, Parada), and DJ (2, AlainaMarie). A duplicate of Barb (Moseph) was among those sequenced. Our new cluster DC phages are closely related, with lengths ranging from 58364 to 59514 and 95-100 genes. All our DC phages have two integrases and some have recognizable immunity repressors. Fireball shares the same serine integrase but has a unique tyrosine integrase and putative repressor. Lamberg, from cluster DD, has a length of 44730 bp and 70 genes. It codes for a toxin, an antitoxin, an integrase, and an immunity repressor. Dexdert has the shortest DE genome (55006 bp) and is

closest to GTE6 and Tiamoceli. The two DI phages, Bock and Parada, have genomes of 50220 and 49909 bp, respectively, and are closely related to each other and all known DI cluster members at the nucleotide level. Both the new DI phages have an immunity repressor, an integrase, and antitoxin and toxin genes. All of our phages had GC content (66.67-67.83%) close to that of the isolation host (67.8%), except DJ cluster phage AlainaMarie (51.29%). The two known other DJ cluster phages (Kerry, Gravy) are similar in gene number and GC% but AlainaMarie is longer (61315 vs 59545-59608) and has several orphans, including near the beginning of the genome. Interestingly, we had difficulty isolating DNA from our *Gordonia* phages, but not from our enterobacteriophages. We switched from column-based extraction kits to a traditional K-Acetate precipitation in isopropanol, with high success. We hypothesize that the burst sizes of our *Gordonia* phages are modest, increasing the challenge of extracting sufficient DNA, though we did not have time to attempt burst size estimates. Finally, we report the development of PhageLog, software that supplies a simple GUI to more easily and consistently format notes on each ORF, for swifter import into DNAMaster. The program checks basic requirements, such as that the ORF length is a multiple of three. Further developments are planned.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #008

Carnegie Mellon University  
Pittsburgh PA

Corresponding Faculty Member: Natalie McGuier (nmcguier@cmu.edu)



Noah Y Lee



Bria K Persaud

### ***Ambrosius and Friends: Identification of novel small-genome and temperate *Arthrobacter* phages***

Manaswitha Edupalli, Mohamed A Eltaeb, Ian Griswold, Peter Han, Elisabeth H Iszauk, Suraj Joshi, Yeonju Kim, Kate Krakopolsky, Victoria Kubyshko, Jae Lee, **Noah Y Lee**, Gjystina Lumaj, Jake Muskovitz, Jiachen Ning, Emery Noll, **Bria K Persaud**, Neyha Shankar, Kevin Shim, Chaitanya Srinivasan, Isabel Yoon, Simone Zhang, Ugne Ziausyte, Jon Jarvik, Javier Lopez, Natalie McGuier

We are exploring the diversity of bacteriophage that infect *Arthrobacter* to improve our understanding of their evolution, their biological impact, and potential applications. We isolated 22 bacteriophages that infect *Arthrobacter sp.* One, KBurrousTX, was sequenced at University of Pittsburgh and was fully annotated. Using the Illumina MiSeq platform and Sanger sequencing, we generated and completely assembled the genomic sequences for 14 of the remaining phage. These genomes were partially annotated. We also completed the sequencing and annotation of Ambrosius, which was isolated in 2013. Sequence alignments, gene content and genome organization allow assignment of 5 phages to cluster AK, 4 to AO2, and one each to AL and AR.

Two of the new phages clearly form a novel cluster with Ambrosius: Gates51 and Wrastor are 98% identical to each other and 88% identical to Ambrosius, but all 3 exhibit little sequence similarity to other known phages, including the other small-genome phages of *Arthrobacter*, *Gordonia* and *Microbacterium*. Their 16 kb genomes have identical gene content and organization, including a putative Tailspike/Lipase protein that is shared with *Arthrobacter* phage Galaxy and is also located between minor tail protein genes and an endolysin. Although the Ambrosius-like phages lack a discernible integrase, they generate stable lysogens that exhibit specific immunity, release infectious particles and contain phage DNA that is detected by PCR. The mechanism of

lysogenization is unknown. However their genomes contain a putative immunity repressor at a location similar to that in the small *Gordonia* CW phages.

The new phage Bashari also produced true lysogens as shown by specific immunity, release of infectious particles upon induction with Nalidixic Acid, and PCR detection of the phage DNA in the bacteria. We could not obtain the complete sequence of Bashari, but it appears to be a novel phage. Consistent with its temperate behavior, one contig contained similarity to the integrase gene of *Microbacterium* phage Min1 and to *Arthrobacter* tRNA –Arg, which might be the integration target. PCR with primers designed against this contig confirmed its presence in the lysogens and in the released infectious particles, whereas it is absent in non-lysogenized *Arthrobacter*. Integrases and temperate behavior are uncommon among *Arthrobacter* phages. It will be interesting to investigate Bashari and the Ambrosius-like phages in greater detail.

Phage-resistant bacteria were also obtained with 8 other bacteriophage, but none were lysogens. The patterns of resistance to all 22 phages were characterized and used for cluster analysis. Some exhibited specific resistance to a particular phage cluster (e.g. AK), but others exhibited more complex patterns that did not map to a specific phage cluster. This information could be useful for elucidating the mechanisms of resistance.

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## 9th Annual SEA-PHAGES Symposium Abstract

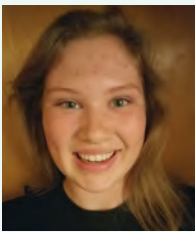
Poster #009

Carthage College  
Kenosha WI

Corresponding Faculty Member: Deb Tobiason (dtobiason@carthage.edu)



Gunnar Goetz



Sarah Schmidt

### ***Investigating Four Gordonia Bacteriophages: Apricot and the Three CR***

Gunnar Goetz, Sarah Schmidt, Sarah Amburn, Juana Carrera, Indirah Conover, Brody Cornell, Dakota Fuss, Hannah Gregory, Alexis Kuhn, David La Rosa, Owen Lewer, Kathryn McKinnon, Cayden Sparks, Emily Vanderploeg, Tyra Wooster, Patrick Zaker, Brittany Niemann, Raven Poirier, Emily Wright, Dianna Bindelli, Anna Pitts, Adam Larson, Brady Sveen, Andrea Henle, Qinzi Ji, Deborah Tobiason

Due to the vast number of bacteriophages in existence, the potential of novel discoveries is high, and every additional bacteriophage contributes new genetic data. Through the SEA-PHAGES program, 78 bacteriophages were isolated at Carthage College during the 2016-17 school year, half of which infect *Mycobacterium smegmatis*, and the other half infect *Gordonia terrae*. Six phages were sequenced, and four Gordonia phages were annotated and further analyzed. The first annotated phage, Apricot, is a singleton and has a genome that is 52195 bps in length with 101 genes and defined ends. It shares the most similarity with cluster DN and DH phage and has two putative Lysin A genes and 10 orphans, one of which has a SecA translocase domain. Apricot is a temperate phage and immunity assays indicate that an Apricot lysogen is immune to infection with itself. Though, it is not immune to infection with the other three annotated phages, Emianna, Jifall16, and Kurt, which belong to cluster CR and are lytic. The cluster CR phages are very similar in regard to genome length and number of genes. Emianna, Jifall16, and Kurt contain a putative HicA-like toxin gene in their genomes. Further bioinformatic analyses of these phages are ongoing to explore the toxin gene, putative repeat sequences, and other sequences of interest. In addition, these four bacteriophages underwent further research using wet lab experiments to learn more about their individual characteristics such as phage particle stability at different temperatures.

College of Charleston  
Charleston SC

Corresponding Faculty Member: Chris Korey (koreyc@cofc.edu)



Renaud Geslain

### ***A novel microarray platform for monitoring the expression of bacteriophage tRNAs during host infection***

Renaud Geslain, Sophia Emetu, Jensen Tomberlin, Christopher A Korey

Transfer RNAs (tRNAs) are small non-coding RNAs expressed in all living organisms. They are essential components of the translation machinery and are responsible for the synthesis of proteins from messenger RNAs. Viruses are obligate parasites that evolved to minimize the size of their genome. As a result, they typically don't encode any elements of the translation machinery and hijack the host's tRNAs and ribosomes for the synthesis of viral proteins. D29 is one of many bacteriophages that infect *Mycobacterium smegmatis*, a nonpathogenic bacterium often used as a model organism to study tuberculosis. Interestingly, D29 encodes five unique tRNA genes specific to Asn, Trp, Gln, Glu and Tyr amino acids. We suspect that these viral tRNAs are expressed during infection to complement the cellular machinery and boost the expression of viral proteins. To monitor bacterial and viral tRNAs expression, we designed and implemented a novel microarray platform. Our approach comprises three steps. First, *M. smegmatis* cultures, infected with D29, are spiked with radioactive orthophosphate; second, labeled total RNAs are trizol-extracted; third, RNA samples are hybridized on in-house printed microarrays and spot signals, the proxy for tRNA levels, are quantified by phosphorimaging. We will present here our tool to measure tRNA expression and discuss preliminary results.

College of Charleston  
Charleston SC

Corresponding Faculty Member: Chris Korey (koreyc@cofc.edu)



Jared Rice

### ***The Identification and Genome Annotation of Four Novel Actinobacteriophage That Infect *Gordonia terrae****

Jared Rice, Ariel McShane, Tommi Nagumo, Jabbarius Ervin, Hayden Tompkins, Cassidy Sullivan, Datayashia Washington, Claudio Miro, Ana Zimmerman, Christopher Korey

Bacteriophages are a type of virus that specifically infect and kill bacterial cells. These phages are being catalogued and studied in order to understand their evolution, in addition to exploring their potential as therapeutics. *Gordonia terrae* is a species of actinobacteria that has been minimally studied, but may have potential uses in environmental conservation through waste remediation. Between the Fall 2016 and Spring 2017 semesters, students collected soil samples from the Charleston area, isolated potentially novel bacteriophages from these samples, and attempted to extract and purify DNA from their phage. DNA from three of these phages—Adgers, Buggaboo, and SuperSulley—was successfully extracted and then sent to the Pittsburgh Bacteriophage Institute to undergo whole genome sequencing, which was done using Illumina sequencing technology. The complete genomes were annotated by a team of six students using various bioinformatic tools, including the genome annotation program DNA Master, the coding potential algorithms Glimmer and GeneMark, BLASTP, and the PEPPER promoter identification program. These characterized genomes will provide insight into the evolution and biological mechanisms of these phages and their *Gordonia terrae* host.



## 9th Annual SEA-PHAGES Symposium Abstract

Poster #012

College of Idaho  
Caldwell ID

Corresponding Faculty Member: Ann Koga (akoga@collegeofidaho.edu)



Alyssa Case



Nicole Jordan

### ***Characterization of *Gordonia* phages asapag and WilliamBoone***

**Alyssa Case, Nicole Jordan, Nilofar Abdul Shukar, Neal Baronian, Emily Bartlett, Jose Cordova, Kirk Doering, Lauren Downer, Miranda Harrington, Brady Nilsson, Jade Rencher, Daniel Sandoval, Laurel Weiss, Elizabeth West, Ann Koga**

In the fall semester we isolated six bacteriophages from *Gordonia terrae* and two phages from *Mycobacterium smegmatis*. We found, through transmission electron microscopy, that the phages all had siphoviral morphology. Because there have been a plethora of mycobacteriophages sequenced, we submitted two *Gordonia* phage DNA samples (WilliamBoone & Asapag) for genome sequencing. WilliamBoone was found to have a genome length of 92,688 bp, and Asapag a much shorter genome length of 55,119 bp. WilliamBoone is a member of Cluster CQ, has a GC content of 61.9%, has eight predicted tRNA genes, and 185 predicted protein-encoding genes, including a programmed frameshift in the tail assembly chaperone protein gene. Although WilliamBoone has a predicted integrase gene, immunity repressor gene and exonuclease gene, we were unable to locate an attB integration site in *Gordonia terrae*. Asapag is a co-founder of a new phage cluster, DN, has a GC content of 63.1%, no predicted tRNA genes, and 101 predicted protein-encoding genes. Five other DN cluster phages were sequenced this year. Asapag has a high degree of nucleotide homology with all five other newly-discovered DN phages. At the right end of the Asapag genome there are several “white” genes. Analysis by HHPred (1) revealed homology of a few of these gene products with previously-described toxin-antitoxin gene products; however the motifs identified are also consistent with transcriptional regulators. More investigation is needed to verify the functions of these genes.

1.Söding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. *Nucleic Acids Res* 33:W244–W248.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #013

College of St. Scholastica  
Duluth MN

Corresponding Faculty Member: Daniel Westholm (dwesthol@css.edu)



Amanda E Rieffer



Melanie M Reese

### ***Analysis of Putative Repressor/Antirepressor Induction System in Cluster P Mycobacteriophages Arib1 and Brusacoram***

Amanda E Rieffer, Melanie M Reese, Matthew A Blow, Daniel J DeLestry, Jacob W Egelhoff, Stephen F Hokanson, Sheila O Iteghete, Maggie M Lenertz, Mitchell A Marinack, Anna F Ribich, Robert S Stenseng, Daniel E Westholm

Cluster P mycobacteriophages Arib1 and Brusacoram were isolated using *Mycobacterium smegmatis* mc2155 as part of the SEA-PHAGES program at The College of St. Scholastica in Duluth, MN. Arib1 has a 46,732 bp genome containing 77 putative genes and 8 promoters. Brusacoram has a 47,618 bp genome containing 78 putative genes. Phylogenetic analysis using MEGA software indicates the closest Arib1 relative is Majeke, isolated 9000 miles away in South Africa, not Brusacoram, which was isolated nearly in the same location as Arib1. Although potential Arib1 lysogens were isolated, the clear plaque morphology for both phages under standard culture conditions suggests a preference for the lytic cycle. Interestingly, an antirepressor gene along with lysogeny-related integrase and repressor genes were bioinformatically identified in both Arib1 and Brusacoram genomes. Phylogenetic analysis of the Arib1 repressor and antirepressor sequences indicated Brusacoram as the closest relative. The lack of agreement between the gene-specific and full genome phylogenies suggests a degree of genetic mosaicism or drift in the cluster P genomes. In order to gain insight into the expression activity of the repressor/anti-repressor genes in Cluster P phages, we performed RNAseq and LC-MS/MS on Brusacoram infections. We infected the host in liquid culture at high MOI and isolated RNA or infected cell pellets at one and 4-hour time points for analysis. At the one-hour time point, the integrase and repressor were the most abundant RNA transcripts expressed from the Brusacoram genome. There was very little anti-repressor transcript expression. However, at the four-hour time point, LC-MS/MS data indicated antirepressor protein expression, but no integrase or repressor protein was detected. Unfortunately, due to experimental and other limitations, we do not have data from both techniques at each time point. In conclusion, our data suggests a rapid induction of the lytic cycle during Cluster P infection through the antirepressor inactivation of repressors required for lysogenic cycle.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #014

College of William & Mary  
Williamsburg VA

Corresponding Faculty Member: Margaret Saha (mssaha@wm.edu)



Destiny Cates



Justin Reed

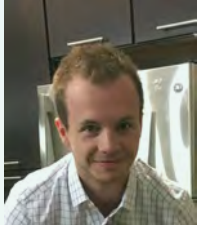
### ***Analysis of Skysand, a Novel CR Cluster *Gordonia* Phage***

**Destiny Cates, Justin Reed, Olivia Allison, Ahlexus D Bailey, Sarah Belay, Monica E Bonilla, Yessica V Bonilla, Danielle S Brown, Ezekial I Carrothers, Joseph Chibueze, Michaela L Davis, Briana L DelaEspriella, Alicia Draper, Jessica A Fleury, Kayla Gibbs, Victoria Guillen, Stephanie Guzman, David Hibbitts, Ivy J Johnson, Autumn Liu, Sarah Jo McGeady, Sarah Modlin, Tanner K Nelson, Meadow E Parrish, Sudip Paudel, Alexandria Pete, Xavier S Burgos, Divinity S Summers, Alexander O Washington, William J Buchser, Mark H Forsyth, Margaret S Saha**

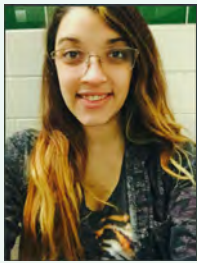
Following over 100 unsuccessful attempts to isolate phages from several species of *Corynebacteria* from a vast array of diverse locations near Williamsburg, Virginia, in the fall of 2016, the William & Mary Phage Lab then switched to a new host - *Gordonia terrae*. Using standard procedures, no phages were isolated via direct plating but twelve out of twenty students easily isolated phages from various campus locations following enrichment. One phage, Skysand, was selected for sequencing and further analysis; Skysand is 67,359 bp in length with 65.5% GC content and is a member of the CR cluster. It is most closely related to the phage Patio with which it shares 97% identity, and based on sequence comparisons, it is possible that Patio and Skysand may constitute a new subcluster. Annotation of Skysand revealed the expected classes of metabolic and structural genes as well as those for infection, and DNA replication, however 31% of Skysand genes have no known function. In addition, we note some interesting findings such as the presence of genes that might encode proteins related to Colicin A and the HicA toxin. Notably, based on Phamerator results, Skysand has 13 genes that are not present in any other phage, thus suggesting that Skysand represents a novel phage.

Collin College  
Plano TX

Corresponding Faculty Member: Bridgette Kirkpatrick (bkirkpatrick@collin.edu)



**Britt M Int-Hout**



**Lydia Flores**

## ***NoodleTree Myoviridae Bacteriophage Annotation Genomic Annotation and Analysis for Genes Indicating Tuberculosis-Related Activity***

**Britt M Int-Hout, Lydia Flores, Wesley R Neuner, Thomas Sposito, Sophia L Hines, Jonathan Lawson, Carole M Twichell, Bridgette L Kirkpatrick**

The Mycobacteriophage, NoodleTree, was discovered in 2016 from soil near the main fountain on the Spring Creek Campus of Collin College in Plano, Texas. After isolation, amplification and imaging, it was found to be a unique Myoviridae bacteriophage that infects *Mycobacterium smegmatis* (mc2155). After DNA extraction, the DNA was sequenced by a team at the Pittsburgh Bacteriophage Institute, and genomic data was made available for annotation. The phage genome has a length of over 150 kbp and is a member of the C1 phage cluster. Upon analysis of the phage genome using DNA Master and its accompanying suite of verifiers, NoodleTree was found to have unique characteristics among viruses that infect *M. smegmatis*. During annotation, we identified several different genes and gene gaps with unknown/novel function.

Our annotations revealed irregular genomic occurrences including missing and altered genes relative to others in the cluster. A large series of genes present in other C1 clusters is absent from NoodleTree's genome. The lack of these genes may hold pertinent information as to if the phage has efficacy in its host choice. Gene 26 (Fig. 4) is a divergent pham in comparison to the other C1 Clusters. HHPRED reads this gene as a part of the phasin-phasin protein family, a family of surface binding proteins. This unique gene may allow NoodleTree to recognize and infect bacteria differently than other C1 cluster phages. Gene 61 is also a divergent gene

compared to other C1 cluster phages. but the function is currently unknown.

NoodleTree infects the target organism, *Mycobacterium smegmatis*. *M. smegmatis* is genetically similar to *Mycobacterium tuberculosis*. *M. smegmatis* shares over two thousand homologous genes as well as the same peculiar wall structure found in *M. tuberculosis*. In analyzing NoodleTree's genome, genes may be identified that specifically target structural and functional aspects of *M. smegmatis* and, therefore, *M. tuberculosis*. In correctly identifying these areas of interest, the overall biological processes of both the phage and target bacteria can be examined for use in several applications including phage therapy, prophylaxis treatment, and oncolytic viral treatments.

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## 9th Annual SEA-PHAGES Symposium Abstract

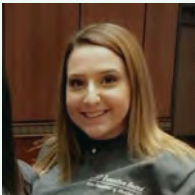
Poster #016

Culver-Stockton College  
Canton MO

Corresponding Faculty Member: Esa Seegulam (mseegulam@culver.edu)



Maryanna K Catrine



Elizabeth Q Hulsey

### ***Isolation and Characterization of the Bacteriophage Jaykayelowell***

Raven S Bugh, **Maryanna K Catrine**, Macie B Crow, **Elizabeth Q Hulsey**, DaJanea A Lloyd, Michael R Pinne, Cheyenne L Roberts, Macie R Spencer, Skyler R Sullens, Madison T Thompson, Sydney M Williams, Muhammad E Seegulam

The objective of this research was to isolate and characterize novel bacteriophage from soil samples. Phage was collected from soil in the Northeast Missouri region, plated to infect a bacterial lawn of *M. smegmatis* mc2155, and subjected to purification in order to identify a novel phage. The presence of plaques in the bacterial lawns was indicative of the presence of bacteriophage in the sample. Morphologically, the plaques were lytic, large, and circular but became consistently smaller and more numerous as plating was repeated with the goal of amplifying the phage population. The DNA was extracted for future analysis. Bacteriophage Jaykayelowell isolated by the 2015 cohort was analyzed. The Jaykayelowell genome was found to be 51,367 base pairs in length and was assigned to cluster A subcluster A4. Among the 88 genes present in the Jaykayelowell genome, several encoded known products including minor tail protein, capsid maturation protease, and membrane domain protein, while almost half of the genes were found to have no known function. The programs used for annotation include DNA Master, Phamerator, Starterator, HHpred, PDB BLAST, and NCBI BLAST.

Del Mar College  
Corpus Christi TX

Corresponding Faculty Member: Daisy Zhang (dzhang@delmar.edu)



Wyatt A Hooks

### ***Using the DnaB Helicase Gene in the Novel Mycobacteriophage 'Wyatt2' to Further Understand M. tuberculosis***

Wyatt A Hooks, Casey L Trevino, John F Ramirez, John R Hatherill, Daisy Zhang

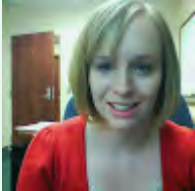
In 2015, it was reported about 1.8 million people died from tuberculosis. Mycobacterium tuberculosis causes infectious tuberculosis. Mycobacteriophages are viruses that infect mycobacterial hosts, such as Mycobacterium tuberculosis (TB) and Mycobacterium smegmatis (M. smeg). In this project we purified, sequenced, and annotated a novel Mycobacteriophage named 'Wyatt2'. 'Wyatt2' is classified as a cluster L, sub cluster L1 with 122 putative genes and 9 putative tRNAs. M. smeg is the host bacteria used to isolate Wyatt2 because it's closely related to TB. Based on GeneMark coding maps, we found that 'Wyatt2' had higher coding potential to TB than M. smeg, which may suggest a closer lineage. We counted 122 genes in total and compared each gene between the M. smeg and TB GeneMark coding maps. In fact, about 96.7% of genes from 'Wyatt2's genome had coding potential with M. tuberculosis while about 73.8% of genes with M. smeg. During annotation of Wyatt2's genome, gene number 68 in the sequence had similar characteristics to DnaB helicase. DnaB functions as a helicase by unwinding DNA. This process starts when DnaA loads a DnaB-DnaC complex onto the DNA. Once the DnaB reaches the replication fork DnaC is released, DnaB then begins to unwind the DNA. The DnaB helicase in Wyatt2's genome has the potential to give further insight of the function of DnaB in TB. Future studies include the mechanism for DnaB, which could potentially create a drug target to stop or hinder the replication of pathogenic, multi-drug resistant TB.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #018

Doane University  
Crete NE

Corresponding Faculty Member: Erin Doyle (erin.doyle@doane.edu)



Erin Doyle

### ***A GENOME IN A DAY: LESSONS FROM THE UPDAWG GENOME ANNOTATION HACKATHON***

Erin Doyle, Catherine Chia\*, Angela McKinney\*\*, Sara Tolsma\*\*\*

\* University of Nebraska-Lincoln, Lincoln NE

\*\* Nebraska Wesleyan University, Lincoln NE

\*\*\* Northwestern College, Orange City IA

A hackathon is an event in which computer programmers, software developers, and others work together to solve a relatively complex problem in a short period of time (often a single day). Students and faculty from Doane University, the University of Nebraska-Lincoln, Nebraska Wesleyan University, and Northwestern College participated in a day-long genome annotation hackathon in which they attempted to complete a single phage genome annotation in a single day. We worked on the cluster A2 phage Updawg, which has a genome length of 53,043bp and 96 ORFs predicted by auto annotation. Additional features of interest included a tRNA gene and a programmed translational frameshift. Students from the four institutions worked collaboratively in cross-institutional groups to annotate assigned sections of the genome and verified their work with faculty members before submitting their sections to be added to the final annotation. The students were able to complete the entire genome annotation during the 5 hour hackathon; however, additional time was needed to finish compiling all of the files for submission. We will discuss student experiences, logistics, and lessons learned (both good and bad!) to help others who may be interested in attempting a similar activity.

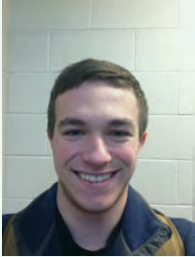


## 9th Annual SEA-PHAGES Symposium Abstract

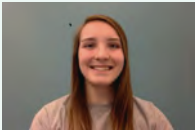
Poster #019

Doane University  
Crete NE

Corresponding Faculty Member: Erin Doyle (erin.doyle@doane.edu)



Nick Iwata



Jade Prochaska

### ***ISOLATION, GENOME ANNOTATION, AND BIOFILM ANALYSIS OF KSQLARED, A NOVEL CLUSTER P MYCOBACTERIOPHAGE***

Nick Iwata, Jade Prochaska, Ashlynn Baker, Hannah Carlstedt, Autumn Hurd, Cassidy Kepler, Jackie Lewis, Kelly Leukens, Cali McEntee, Kelsey Snyder, Kali Ulrich, Erin Doyle

The overuse of antibiotics and the resulting increase in antibiotic resistant bacteria has become a major concern in the medical community. One proposed solution is the use of bacteriophage (phage) that infect and kill specific bacteria. Phage have a unique benefit for possible treatment of infections because the phage can adapt to the mutations in the bacteria. To be able to research such possible uses for phage, science must first learn about their genomes to understand more about how they work. We isolated the phage Ksquared from soil on Doane University's campus using the SEA-PHAGES protocol. Ksquared infects *Mycobacterium smegmatis*, a close relative to *Mycobacterium tuberculosis*. Ksquared is a lytic siphovidae. Mycobacteriophage Ksquared was selected for annotation due to it being a part of the rare P cluster and P1 subcluster. With the genome sequenced, we were able to annotate the genome of Ksquared. 41 of Ksquared's annotated genes were assigned functions. Endodeoxyribonuclease was one of the unique functions found by HHPRED; however, further testing would be needed to assign such a specific function as endodeoxyribonuclease. Additionally, Ksquared shares a small region of similarity with the singleton phage Pine5. Overall most of the functions found and assigned when Ksquared was compared to these phages: Brusacoram, Fishburne, Phayonce, and Tortellini. In the future, we will also test Ksquared's effectiveness against bacteria grown as biofilms, which can increase antibiotic resistance.

Dominican College of Blauvelt  
Orangeburg NY

Corresponding Faculty Member: Bernadette Connors (bernadette.connors@dc.edu)



**Christina Joseph**

## ***The Mycobacteriophage of Dominican College: Direct Isolation of Fifteen Siphoviridae from Soil and Water***

**Christina Joseph**, Melody Scrudato, Linda Dantico, Annie Segura, Yunia Alvarez, Destiny Lora, Rebecca Birmingham, Tara Carney, Cynthia Castro, Kayla Johnson, Michael Panis, Abel Rosario, Raymond Arno, Marie Louis, Liana Rosario, Marcos Pierce, Samuel Hyde, Mariela Martinez, Miriam Mendoza, Angela Koza, Alexandria Ledgerwood, Alexis Montoya

In conjunction with the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES), students at Dominican College isolated and purified mycobacteriophages from the surrounding campus environment by way of direct isolation. Using protocols outlined in the Phage Discovery Guide, fifteen novel lytic phages were purified using *Mycobacterium smegmatis* mc<sup>2</sup>155 as the host. Transmission electron microscopy and restriction endonuclease digestion were completed in order to determine both morphotype and cluster to which each belonged. In addition, incubation on the Xeno lysogen tested each phage's ability to infect. All purified phages belonged to the siphoviridae family, and all but four phages infected Xeno at an EOP of 1. One of the resulting novel lytic phages, Mattes, was sequenced and classified in F1 subcluster. Its genomic sequence had 61.5% GC content and showed 99% similarity to *Mycobacterium* phage James. The remainder of the purified phage were sequenced *en masse* and subjected to DOGEMS analysis. Four phages were identified, one of which (Joselito) yielded a full sequence. It was determined to have 99% similarity to the A11 phages Lucivia and Flaverint, with 63.7% GC content and one tRNA. Joselito infected Xeno at EOP of <1, and presented with both lytic and lysogenic plaques. To create lysogens of Mattes and Joselito, mesas were allowed to form at 30°C, assayed by the patch test, and streaked three times to remove endogenous phage. A patch test was repeated, and immunity to the other phages was determined.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #021

Drexel University  
Philadelphia PA

Corresponding Faculty Member: Susan Gurney (smg366@drexel.edu)



**Pauline M Good**



**Andrew D Jurgielewicz**

### ***B1 is for is for Bacteriophage***

**Pauline M Good, Andrew D Jurgielewicz,** Lavanya S Aluri, Dharman Anandarajan, Jamison C Beiriger, Raviteja Bethamcharla, Niteesha Betini, Shivangi D Bhatt, Swetha Chengalvala, Nicole E Cox, Bela P Delvadia, Aishwary S Desai, Andrew M Devaney, Brenna K Doyle, Arden O Edgerton, Matthew C Erlich, Kevin C Fitzpatrick, Esha A Gajjar, Anjali Ganguly, Ramnik S Gill, Madeleine G Goldman, Nishtha Gupta, Leila M Haddad, Esther J Han, Shelby Jain, Andrew Jiang, Devneet K Kainth, Jawhara M Karam, Mallika Kodavatiganti, Sinja J Kriete, Catherine E MacDonald, Josh P Maret, Ashley E Mathew, Sprikena Nako, Maanasa Natrajan, Nusrat M Nishu, Ayush A Parikh, Nirali Patel, Pooja D Patel, Shivani Patel, Kaustav Patra, Daechaton Pumpuckdee, Karima K Rai, Anshul Ramanathan, Arghyadeep Sarkar, Brianna L Schaffer, Priyanka Shah, Ravi K Tata, Andrew H Tawfik, Bhavya T Thuremella, Justina Toma, Trinh Tran, Shika Veera, Vamsee K Vemulapalli, Trevor V Vidas, Katy S Vieira, Gayathri Vijayakumar, Tru A Walor, Clara R White, Brianna M Wong, Shu I Zhao, Ritu R Dalia, Alison E Moyer, Susan M Gurney

This year Drexel University freshman undergraduate students isolated 56 bacteriophages that infect *Mycobacterium smegmatis mc2 155*, from soil samples. Of these phages 5 were sequenced and their genomes annotated. All 5 phages belong to the B1 cluster. 71.4% of the bacteriophages annotated by Drexel University to date (10 of 14 phages) belong to the B1 cluster, compared to the national average which is 11.8%. Our students spent 10 weeks working on independent projects to advance their understanding of the characteristics of the B1 cluster phages isolated at Drexel. Vamsee Vemulapalli compared the distribution of bacteriophage clusters isolated by SEA-PHAGES students across the United States of America. He then compared this distribution data against regional temperature and soil type to determine if there were any correlations between geographic location and cluster identity. Maanasa Natrajan compared the phages within the sequenced B1 cluster phages from this year and identified similarities across the number of their genes, GC content and nucleotide length. The other students performed wet lab experiments to characterize the phages'

growth patterns when exposed to different conditions. These conditions included testing the phages ability to infect different host cells and the study of their plaque morphologies under stressful conditions (e.g. acidic, alkaline pH and higher glucose concentrations).

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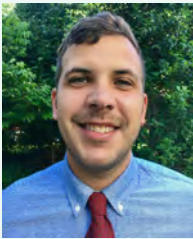
## 9th Annual SEA-PHAGES Symposium Abstract

Poster #022

Durham Technical Community College  
Durham NC  
Corresponding Faculty Member: Steven Leadon (leadons@durhamtech.edu)



Roselyn Barahona



Michael Herrera

### ***Phage Pharming: Characterization of Two Novel Phages, Kimchi and Glexan, Isolated in the Gardens of North Carolina***

Roselyn Barahona, Michael Herrera, Nanci Becquer-Ramos, Emma Furman, Natalya M Moreno, Khanh H Nguyen, William H Stevenson, Nicholas Toma, Alan Vedder, Katie S Bolling, Marie P Fogarty, Steven A Leadon

Increasing use of antibiotic drugs has raised concerns of antibiotic-resistant bacteria, prompting research into alternative therapies such as utilization of bacteriophages to fight infection. During the fall of 2015 and 2016, the Durham Tech Phage Hunters lab discovered two novel mycobacteriophages, Kimchi and Glexan, by collecting soil samples and using *Mycobacterium smegmatis* as a host to isolate them. Kimchi was found near a vegetable compost pile in a garden in Durham, NC. Gene annotation and analysis characterized Kimchi as a Cluster E phage with 75829 base pairs, 63% GC content, encoding two tRNAs, and a Siphoviridae morphology. Glexan was found in a recently fertilized garden bed in Chapel Hill, NC. Gene annotation and analysis characterized Glexan as a Cluster E phage with 76498 base pairs, 63% GC content, encoding two tRNAs, and a Siphoviridae morphology. To date, there are 108 Cluster E phages listed in PhagesDB. After completing gene annotation, bioinformatic analyses were performed to further characterize these phages to identify start site preferences, promoters and terminators, transposable elements, and putative recombination sites. Both Kimchi and Glexan were found to prefer ATG as a start site, accounting for 63% of the total start sites. Sigma-70 promoter prediction and subsequent WebLogo generation showed a -35 consensus sequence of TTGACA and a -10 consensus sequence of TATAAT for both Kimchi and Glexan. ARNold analysis predicted the best terminator sequence in Kimchi and Glexan and characterized it as a Rho-independent stem-loop terminator. Phagesdb BLASTn and Gepard analyses generated sequence alignments of Kimchi to similar phages to reveal possible insertion sequences or transposons. Additionally, the consensus Shine-Dalgarno sequence for Glexan

was identified by aligning upstream sequences of all the genes in Glexan and searching for matching base-pair sequences. The presence of an integrase gene in both Kimchi and Glexan led us to look for possible sites of recombination between the phages and *M. smegmatis*. Based on a genomic comparison of Kimchi and Glexan with *M. smegmatis* using NCBI BLASTn, a putative attP site was identified for each phage: between base pairs 37643-37660 for Kimchi and between base pairs 37982-37999 for Glexan. Our bioinformatic inquiry identified 13 transmembrane proteins in Kimchi and 12 in Glexan. Two of the proteins identified in both Kimchi and Glexan were the Tape Measure protein and Holin. The Tape Measure protein contained four hydrophobic regions, each of which was 23 amino acids long, while Holin contained only two hydrophobic regions that were each 23 amino acids in length. These experiments provide further insight into members of Cluster E phages and contribute to a more thorough understanding of mycobacteriophage evolution and genetic diversity.

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The Evergreen State College  
Olympia WA

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Sophie Harrison

### ***Isolation and Analysis of Cluster Q Phage Ein37 and F1 Phage Plumbus***

Daniel Devonshire, Sophie Harrison, Cody Allison, Samantha Altheide, Brianna Bordwell, Mandy Chen, Adrienne Chitwood, Tokala Christensen, Carollyne Coulson, Nichole Criss, Melinda Dauley, Christopher Davis, Kyle Dobbs, Jacob Durham, Michael Espy, Jarred Figlar-Barnes, Parke Funderburk, Evan Furrer, Brianna Gage, Daniel Gilberts, Andrew Glassbrook, Kyle Grosten, Jenny Green, Annelise Hooper, Casey Irwin, Coleman Kaestner, Katherine Kallus, Nolan Knaus, Tamara Koledin, Randolph Kraus, Stacia Krause Ledbetter, Tessa LaLonde, Madesyn Lockwood, Anthony Lopez, Kody Ludwig, Nacia Magass, Trevor McCliment, Bryanne McNamara, Robert Munoz, Micah Needham, Melyssa Noci, Taylor Nowotny, Luke Ogden, David Parker, Habib Quaraishi, Olivia Parks, Kaden Phillips, Rian Plastow, Justin Roberts, Shayna Rossiter, Breck Roundtree, Candace Saunders, Marissa Scoville, Alexea Seil, Petal Shohadai, Keelan Smith, Sean Staub, Charles Thompson, Steven Vananzo, Amber Vidal, Jenny von Henkelman, Crystal Whitford, Chandler Williams, Clarissa Dirks, Alberto Napuli, James Neitzel

The student participants in our third cycle of the SEA-PHAGE project were again members of the interdisciplinary program *Introduction to Natural Sciences*, a year-long, full-time learning community with integrated instruction in biology, chemistry, and science process skills. During the fall quarter students collected and purified phages using enrichment cultures of *Mycobacterium smegmatis* mc<sup>2</sup> 155 at 37 C. This year 23 phages were isolated from local soils, purified, and entered into the PhagesDB collection. Compared to prior years, we had a lower percentage of successful isolations. This set of phages had their DNA purified and analyzed by restriction enzyme digestion and gel electrophoresis. Successful DNA extractions were completed for most of the phages, although some difficulty in dealing with DNA stability was encountered. DNA quality and restriction enzyme experiments were used to select genomes for sequence analysis. Phages were also analyzed by transmission electron microscopy after negative staining with uranyl acetate. This resulted in clear images of all of the isolated phages, which appeared to be *siphoviridae*.

The phages sequenced and analyzed were Ein37 and Plumbus. DNA from these phages was sequenced using the Illumina process at the Pittsburg Bacteriophage Institute. The sequence of Ein37 revealed a 53,748 bp linear double stranded DNA genome with a sticky fourteen bp 3' overhang and with a GC content of 67.4 %. Analysis of the sequence of this phage confirmed that it was a *siphoviridae* in the Q cluster. BLASTn results

showed that Ein37 had a 99% sequence identity with the other 7 members of cluster Q, indicating a high degree of similarity in this group, with almost half of the predicted genes being Phams unique to this cluster.

The genome of Plumbus is a 54,468 bp dsDNA with a sticky ten bp 3' overhang and a GC content of 61.1%. BLASTn results indicated its closest relative was Cluster F1 phage Kimberlium. Both genomes were analyzed for potential protein coding open-reading frames using Glimmer and GeneMark, and protein functions were predicted by BLASTp and HHPred, as well as examining synteny with related phages. Preliminary results suggest the presence of 86 protein coding genes in Ein39 and 106 in Plumbus. We identified no tRNA or tmRNA genes in either phage. Further work is being conducted to identify and confirm all protein coding regions and to identify functions for predicted protein products.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #024

Fayetteville State University  
Fayetteville NC

Corresponding Faculty Member: Kristen Delaney-Nguyen (kdelaney@uncfsu.edu)



Lena Franklin



Ismael Hernandezvillasuso

### ***Isolation and Characterization of Mycobacteriophage Cornie, the Lone Member of the New F5 Subcluster***

Lena Franklin, Ismael Hernandezvillasuso, Alan Ardin, Rebecca Galloway, Justin Lugo, Samantha Selix, Ambria Thomason, Kristen Delaney Nguyen

Bacteriophages are the most numerous microbes in the biome and display massive genetic diversity. Thirteen phages capable of replication in *Mycobacterium smegmatis mc2155* were isolated in the Sandhills region of North Carolina. Three phages (Cornie, FrayBell, and Leogania) were sequenced at the Pittsburgh Bacteriophage Institute. All three of the sequenced phages were novel. Fraybell demonstrated myoviridae morphology and the remainder were siphoviridae. Cornie is of particular interest as it is the lone member of the new subcluster F5. The plaques of Cornie have a slight comet tail appearance when grown in *M. smegmatis mc2155*. Many of the encoded proteins had higher homology to bacterial genes than the genes of other phages. Cornie appears to be a lysogenic phage due to its turbid plaques and the presence of lysogeny-associated genes such as an integrase, an immunity repressor, a CRO repressor, and antirepressor. Continuing work will involve seeking related phages from the same geographic region using a Cornie lysogen.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #025

Florida Gulf Coast University  
Fort Myers FL

Corresponding Faculty Member: Scott Michael (smichael@fgcu.edu)



Sara Feith



Dana Indihar

### ***A Bioinformatic Investigation of *Gordonia terrae* Phage Diversity in Southwest Florida***

Sara Feith, Dana Indihar, Lauren Alvaro, Kayla Baker, Ian Baxter, Matthew Brown, Kathleen Driscoll, Johan Elrubaie, Michael Kratz, Vanessa Knoch, Kathryn Koirtzohann, Andrew Lear, Kaitlin Lindblom, Emily Marcus, Molly Murphy, Caitlin O'Brien, Rebecca Sensor, Victoria Swift, Samuel Sherman, Kelsey White, Samantha Wills, Brandon Ashley, Samantha Gatt, Carley Gray, Sara Lohbauer, Taylor Power, Kimberly Rosales, Brittany Sisson, Sharon Isern, Scott Michael

Florida Gulf Coast University students isolated fifteen phage using the host *Gordonia terrae* 3612. Of the three phage that were sequenced, two, DatBoi and Mollymur, are cluster DL siphoviridae and one, Skog, a cluster DO myoviridae. Skog is particularly interesting since it has the longest genome of all the sequenced *G. terrae* phage (152,435 bp). We compared our phage genomes to their closest relatives, Pupper and SCentae from cluster DO, found by the University of Pittsburgh, and Bantam from cluster DL, found by University of Wisconsin-River Falls, as well as other more distantly related phage. We analyzed the genomes from all *G. terrae* phage to look for the absence of 6 bp restriction sites. We failed to find any single restriction sites that were missing from all *G. terrae* phage genomes, suggesting that *G. terrae* may not have an active restriction enzyme defense system. This may help explain why *G. terrae* phage are so diverse. To investigate the mosaic nature of phage genomes, a phylogenetic analysis of capsid and immunity repressor genes from all *G. terrae* phage revealed that individual genes do not always evolve similarly, but show evidence of horizontal gene transfer. We discovered that genes with high ratios of amino acid change, quantified by the nonsynonymous to synonymous ratio (Ka/Ks), across the genomes of DL phage Mollymur, DatBoi, and Bantam occurred in clusters towards the 3' end of the genome. The majority of genes with high Ka/Ks values were DNA binding proteins, suggesting that transcriptional regulation might be under strong evolutionary selective pressure. To study lysogeny in our new DL phage, we identified several potential attP sites upstream of the tyrosine integrase

gene. However, further inspection of the *G. terrae* host genome did not reveal any corresponding attB sites. Based on a recent paper describing communication systems in temperate Bacillus phage, we identified possible communication peptides within lytic as well as temperate phage that infect *G. terrae* and other Actinobacteria hosts. Overall, we found that Southwest Florida has an abundance of *G. terrae* phage with diverse and interesting genomic properties.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #026

Florida International University  
Miami FL

Corresponding Faculty Member: Mauricio Rodriguez-Lanetty (rodriguezlanetty@gmail.com)



Richard Suarez



Isabel Maria

### ***Bacillus Phages Wooty and Phamous Reveal Abnormal Genome Architecture and Potentially Selfish Genetic Elements***

Richard Suarez, Isabel Maria

Bacteriophages are viruses that utilize specific bacteria as their hosts. The study of bacteriophage genomics is important for understanding the effects that these viruses have on their host. Functional annotations also contribute to the classification of phages and identification of potential virulence factors, which can in turn assist in the identification of bacteria. Wooty is a *Bacillus cereus* phage with a genome that is 165,832 base pairs long, an unusually large genome among phages including phage Phamous with a genome that is 51,310 base pairs long. Given that half of Wooty's open reading frames were in the forward direction and the other half were in the reverse direction, in addition to the presence of eighteen tRNAs, it was hypothesized that Wooty might contain two intact phage genomes. Wooty was most similar to *Bacillus* phages BM10 and Bcp1 based on whole genome BLASTs and individual gene BLASTs. PHAST revealed that all three genomes were composed of multiple regions identified as distinct, intact phage genomes, indicating that Wooty, BM10, and Bcp1 share a largely conserved genomic architecture. Notably, of the two hundred and sixty-two ORFs examined in Wooty as well as the one hundred and one ORFs in Phamous, three putative HNH endonucleases were found in Wooty and five in Phamous. HNH endonuclease is a type of enzyme that creates "nicks" (a single-stranded cleavage) in the genomic DNA and functions at specific recognition sites along the genome due to the DNA-recognition domains in addition to the HNH motif allowing for the high sequence specificity. It also

works alongside other components to form selfish genetic elements—these enhance their own transmission relative to the rest of an individual’s genome, but are neutral or harmful to the individual as a whole. These elements are commonly found in phage genomes as they serve as a form of defense, which may explain why phage defense systems are so diverse. The examination of the function, associations and mechanisms of HNH endonucleases allow us to better understand their persistence in a variety of biological systems.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #027

George Mason University  
Fairfax VA

Corresponding Faculty Member: Anne Scherer (aschere2@gmu.edu)



**Josef Mang**



**Rebecca Munday**

### ***Isolation and Characterization of Two Novel Mycobacteriophages at George Mason University***

Mona-Leigh Allen, Jessica Anang, Felicia Bedillo, Jacob S Camden, Victoria Choe, Ahmad Elmohtaseb, Sienna D Frank, Karina Hinrichs, Chelsea N Horton, Hufsa Khan, Atir Khokhar, Jessica Liu, Amir F Maghsoudi, **Josef Mang**, Fabio J Martinez, Sofia N McDaniel, **Rebecca Munday**, Tiffany H Quach, Jacob Smith, Masooma Zainab, Reid Schwebach, Anne Scherer

Here we describe two novel Siphoviridae bacteriophages that were isolated using the enrichment method. Both phages were isolated from mulch samples on the George Mason University campus in Fairfax, VA using *Mycobacterium smegmatis* mc2155 as the host bacterium. Both genomes were sequenced using Illumina sequencing at the Pittsburgh Bacteriophage Institute.

One of the phages isolated, Easy2Say, was found to be a Cluster E phage, which currently has 84 members. Easy2Say has a genome containing 75,598 bp with a GC content of 62.9%. Several structural proteins were identified, including a major capsid protein, a major tail subunit, and 3 minor tail proteins. Interestingly, Easy2Say contained a beta-lactamase gene which normally confers resistance to penicillin and other beta-lactam antibiotics in bacterial cells. Also identified were several enzymes involved in destruction of the bacterial host cell wall (Lysin A, Lysin B, and a holin protein). We will also describe a -1 frameshift present in the tail assembly chaperone gene preceding the tapemeasure gene.

Our second phage, Phareon, is in subcluster B1, which currently has 161 members. Phareon has a genome containing 68,040 bp with a GC content of 66.5%. Proteins involved in DNA packaging were discovered, including both large and small terminase subunits. At least 4 genes appear to encode minor tail proteins, and

were clustered together downstream of a tail assembly chaperone protein. Genes for replication were discovered, including DNA polymerase and helicase. Phareon also contained genes for destruction of the bacterial host cell wall, namely Lysin A and Lysin B. In addition, there were genes for transcription and translation, such as RNA polymerase, tRNA ribosyltransferase, and an elongation factor.

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Gonzaga University  
Spokane WA

Corresponding Faculty Member: Marianne Poxleitner (poxleitner@gonzaga.edu)

## ***Gonzaga Takes on the Sensitivity, Host Range, and Xeno Challenges***

**Ian McGowen, Baily Luoma, Ashley Beausoleil, Elizabeth Grainy, Jerry Sicalo, Cyla Sparks, Vina Tran, Kirk Anders**

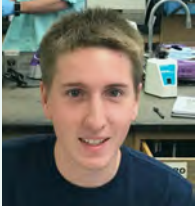
For two semesters students in Biology 405: Advanced Phage Biology, aka Phancy Phage Lab, worked on three separate phage projects. One group investigated lysogen sensitivity in K Cluster phages. Lysogens of Gonzaga phages SamuelLPlaqueson (K1), DrHayes (K1), Cain (K6), and Taquito (K4) were tested for homos and heteros immunity against Ks phages and the phage lysates from the Host Range Challenge. Adephagia, a K1 phage, was able to infect the Cain (K6) lysogen even though other K1 phages were not. Another team chose *Microbacterium testaceum* and *Brevibacterium fuscum* for the Host Range Challenge. In addition to testing the challenge phages for the capacity to infect the hosts, each bacterial strain was tested for the ability to grow in different media including TGY, 7H9, and PYCa. The final group of Phancy Phage students attempted the Xeno Challenge and tested over 100 Gonzaga phage lysates on the Xeno lysogen.



## 9th Annual SEA-PHAGES Symposium Abstract

Poster #029

Hampden-Sydney College  
Hampden-Sydney VA  
Corresponding Faculty Member: Mike Wolyniak (mwolyniak@hsc.edu)



Dakota M Reinartz



Richard W Morgan

### ***Isolation and Genomic Characterization of Bacteriophage Thespis***

Dakota M Reinartz, Richard W Morgan, Michael J Wolyniak

Thespis is a *Mycobacterium smegmatis* bacteriophage that was isolated from a soil sample taken from the campus of Hampden-Sydney College. After isolation and purification of the phage occurred, we sent the phage to have its genome sequenced. Upon receiving the sequence of the genome, it was annotated using the DNA master software. Annotation of the Thespis genome was done by multiple students in our fall Genomics and Bioinformatics course. We found that Thespis has a total of 47,619 basepairs that encode 78 genes. Also it was found that this bacteriophage was very similar to bacteriophage Brusacoram that was isolated and found at the College of St. Scholastica in Duluth, Minnesota. Genes that students found interesting were investigated through BLAST and through primary literature to learn what those genes might function to do in the bacteriophage. Gene 30 and 31 of the Thespis genome were two genes that were investigated further. Gene 30 was found to code for an integrase and 31 was found to code for an immunity repressor. Also towards the 3' end of the Thespis genome many DNA methylase genes were identified. These methylases probably have a regulatory function during the bacteriophage infection. These could be useful genes to look into because of their function as possible regulators in the lifecycle of Thespis. Thespis was found to have lysogenic capabilities based on the genes found and to have regulatory genes present like the DNA methylases in its genome.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #030

Hope College  
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Corresponding Faculty Member: Aaron Best (best@hope.edu)



Madelyn Orndorff



Jacob Peecher

### ***Establishing Mycobacteriophage Cluster AA – Genomic Analyses of Settecandela and Phrappuccino***

**Madelyn Orndorff, Jacob Peecher, Daniel Setteceri, Brandon Bonilla, Alicia Bostwick, Carmen Chamberlain, Diana Davis, Vanessa Drust, Megan Grimes, Julia Keisling, Taylor Lombard, Sydney Les, Anna Lunderberg, Maddison Prebenda, Skylar Sundquist, Veronica Vance, Alexandra Wyeth, Cassandra Harders, Shannon Smith, Joseph Stuke, Aaron Best**

Eighteen new mycobacteriophages were isolated from soil samples collected around the state of Michigan and parts of the United States. All phages were capable of infecting *Mycobacterium smegmatis* and were isolated through either enrichment or direct plating at 32°C or 37°C. A variety of plaque morphologies were produced based on size, shape, and clarity; both lytic and temperate phages appear represented in this collection. Three phages, Opia (Cluster B2), KashFlow (Cluster J) and Settecandela (Cluster AA), were selected for complete genome sequencing and comparative genomic analyses. We focus here on comparative analyses of Settecandela. The predominant plaque produced by Settecandela at 37°C was 1 mm in diameter, and took 48 hours to appear. The complete genome sequence for Settecandela revealed a relationship to the singleton, Phrappuccino, discovered by the Hope College phage class in 2016. The genome of Settecandela is 145.2 Kb, 67.4% GC, and contains 224 genes. The close relationship with Phrappuccino leads to the formation of a new cluster of mycobacteriophages, Cluster AA. The two genomes are identical over much of their length, with the exception of an approximately 9000 bp region in Settecandela containing 26 genes. The Settecandela-unique region begins near the 5' end of gp184; the first 75 nucleotides of this gene are identical with gp183 of Phrappuccino at which point the sequences diverge. Settecandela gp184 encodes a protein of unknown function that is found in *Mycobacterium*, *Rhodococcus* and *Gordonia* bacterial species. The highest BLAST hit

(full length, e-value  $6 \times 10^{-108}$ ) is to a protein with no known function from *Mycobacterium koreense*, a slow growing mycobacterium isolated from a patient with pulmonary dysfunction. Three genes were assigned functions in the unique region; 16 of the genes are currently orphans. The remaining 9 genes are members of phams found in phages from multiple mycobacteriophage clusters. At the end of the unique region, the Settecandela genome contains a full-length homologue to Phrappuccino gp183 (the start of the unique region), with sequence variation occurring in the first 62 nucleotides of the gene. Plaque morphologies of the two phages differ, with Settecandela displaying a comet morphology for some plaques. There is strong evidence at the morphological (Myoviridae) and genomic levels for a relationship of Cluster AA phages to Cluster C phages. Despite this relationship, Cluster AA genomes do not carry any tRNA genes. Additionally, Cluster AA genomes contain homologues to *Rhodococcus* phage E3 and to multiple actinobacterial genera, suggesting the potential for a broadened host range.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #031

Howard Hughes Medical Institute  
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Corresponding Faculty Member: Viknesh Sivanathan (sivanathanv@hhmi.org)



Aleem Mohamed, Ilzat Ali, Emily Davis

### ***Characterization of Actinobacterial Hosts for Phage Isolation***

Ilzat Ali\*, Emily Davis\*, Aleem Mohamed\*, Priscilla Kobi, William Biederman, Vic Sivanathan

\* University of Maryland, Baltimore County, Baltimore MD

The scientific goal of SEA-PHAGES is to explore the biodiversity amongst bacteriophages. As we expand from isolating and studying mycobacteriophages to actinobacteriophages, the careful “piloting” of new actinobacterial hosts can facilitate this process. Here, we present data from piloting 3 actinobacterial hosts, *Arthrobacter sp.*, *Gordonia terrae*, and *Microbacterium foliorum*. This data is presented in the context of other *Actinobacteria*, namely *Microbacterium smegmatis* and *Streptomyces griseus*, the latter with data provided by Lee Hughes of the University of North Texas. It is our hope that the data presented will encourage and facilitate the adoption of these *Actinobacteria* as hosts on which to isolate and study actinobacteriophages.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #032

Howard University  
Washington DC

Corresponding Faculty Member: Mary Ayuk (mary.ayuk@howard.edu)



Raju Sagar



Erin Winrow

### ***Characterization of Mycobacteriophages from the Myoviridae and Siphoviridae Morphotypes***

Taylor Burroughs, Desiree Butler, Brittany Grossi, Alexis James, Giordanne Logan, **Raju Sagar**, Kiara Thomas, Raines Warren, Jada Watts, **Erin Winrow**, Students of HU- PHAGES 2016-2017, Michelle L Fernando, Aliza Ibad, Esohe Irabor, Gretchen Johnson, Jerome Oliver, Michael Smith, Leon Dickson, Adrian D Allen, Winston Anderson, Mary A Ayuk, Leon Dickson, Broderick Eribo, Ayele Gugssa, Courtney Robinson

**Introduction:** Bacteriophages are obligate, intracellular parasites that infect bacteria. They are studied for purposes which include viral ecology, epidemiology, as tools in molecular biology, and in the development of therapeutics. The foci for the 2016-2017 SEA-PHAGES Program at Howard University, were to: (a) investigate diversity of phages that infect *Mycobacterium smegmatis* mc2155, and (b) evaluate phage sensitivity/insensitivity—immunity testing using the aforementioned host.

**Methods:** 100 bacteriophages were isolated from soil samples collected from different locations on the campus of Howard University. All phages were derived from enrichment culturing and purified using standard protocols. DNA was purified, quantitated, characterized and representative samples sequenced using Illumina Sequencing. Sequenced genomes were then annotated using various in-silico programs which include DNA Master. Furthermore, all phages were utilized for identifying patterns of phage sensitivity/insensitivity—immunity testing using wildtype *M. smegmatis* mc2155 and generated lysogens.

**Results/Conclusions:** Data analysis indicate that sequenced phages can be divided into the following four clusters: B (phages Coffee, Luckymarjie, and PLmatters (as well as its duplicate, JulesWinnfield); C (Atlantean and duplicate Doodle 1); G (Renaissance), and Q (Webster2). Coffee's (sub-cluster B2), genome is circularly permuted with length of 67481 bp, GC Content of 69.0%, and belongs to family, Siphoviridae. Coffee is 99% similar to mycobacteriophage Faze9, which was isolated in Scranton, PA. Like Coffee, phage LuckyMarjie, (subcluster B1), belongs to the Siphoviridae family, has a circularly permuted genome with length (bp) 68075,

and GC content of 66.5% . Luckymarjie is 99% similar to phage “Roy17”, which was isolated from the aforementioned location. PLmatters (subcluster B1), a temperate phage, has a circularly permuted genome with length (bp) 68318, and GC content of 66.5%. PLmatters is 99% similar to mycobacteriophage Phunky, which was isolated in Philadelphia, PA. Like all previously described phages, Atlantean (sub-cluster C1) has a circularly permuted genome (154553 bp), with GC% of 64.7. Atlantean is 99% similar to phage Momo, an isolate from Pittsburg, PA, and belongs to the Myoviridae family. Renaissance (G1) has genome length (bp) of 41879, with 11 bp 3' sticky overhang genome ends (CCCCATGGCAT). Renaissance has GC content of 66.6%, belongs to family Siphoviridae, and is 99% similar to mycobacteriophage OctaviousRex, isolated in Newburgh, NY. Like Renaissance, Webster2 is a member of family Siphoviridae, with genome length (bp) 53746, and GC content of 67.4%. However, the 3' sticky overhang is 14 bp with sequence TAAGCCGCGGGTA. This phage is 99% similar to Mycobacterium phage LilHazelNut, isolated in Dahlonga, GA. In addition, preliminary data from sensitivity/insensitivity testing suggests that several of these phages are temperate.

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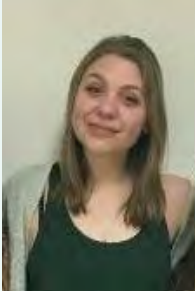
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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #033

Illinois Wesleyan University  
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Corresponding Faculty Member: Rick Alvey (ralvey@iwu.edu)



Zoephia Laughlin



Caroline Marchi

### ***Isolation and Analysis of Mycobacterium smegmatis J and S Cluster Phages Squint and Tesla***

Zoephia Laughlin, Caroline Marchi, Marissa Alcalá, Alecia Beagles, Janna Fitzgerald, Tulasi Jaladi, Jessica Keen, Colin Page, Bailey Reichert, Morgan Reich, Andria Talavera, Samantha Ziomek, Richard Alvey, David Bollivar

During the fall semester of 2016 at Illinois Wesleyan University, fifteen *Mycobacterium smegmatis* strain mc2155 (M. smeg) and nine *Rhodobacter capsulatus* strain YW1 (RC) bacteriophages were isolated. Although each of the five sequenced RC phages from the class was found to be in the same cluster through genome sequencing analysis, specific M. smeg phages, Squint and Tesla, were further analyzed because of their unique qualities recognized during the isolation and purification processes. Squint appeared to be unrelated to any other phage in the class as shown through lysogen formation and immunity testing, and Tesla presented small plaques after being discovered through the direct plating method. After these phages were sequenced at the University of Pittsburgh and annotated by the class, they were found to be members of two relatively rare clusters, J and S. Squint was found to be a member of the J cluster, which until then only had eleven members. Tesla was found to be an S cluster phage, a group comprised of only three phages. Once Squint and Tesla were sequenced and annotated, we could compare the evolutionary relationship existing between the genome of

these bacteriophages and the other phages in the J and S clusters. The discovery and analysis of these J and S cluster bacteriophages is a step towards a greater understanding of phage diversity.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #034

Indian River State College  
Fort Pierce FL  
Corresponding Faculty Member: Tom D'Elia (tdelia@irsc.edu)



Sara Sewelson



Emma Floyd

### ***Expanding diversity and host range of mycobacteriophage***

Sara Sewelson, Emma Floyd, Rafael Cancino, Chris Checo, Anthony Cruz, Laura De Castro, Courtney Gough, Shawna Holmes, Kaleigh Newman, Anthony Thompson, Helen Wiersma-Koch, Tom D'Elia

Cataloguing bacteriophage diversity, and the host range they infect, is important for increasing our understanding of the large and dynamic phage population. The Actinobacteriophage Database is extensive, with 10478 phage isolated from 13 genera of actinobacteria. Of these, 1988 phage have been sequenced. By far, the most extensive number of phage has been isolated and sequenced from *Mycobacterium smegmatis* (1358). In order to increase chances of isolating novel and diverse bacteriophage that infect *M. smegmatis*, enrichments were performed at 42°C. By using this approach, we identified two new phage, Rubeelu and Exilir, belonging to clusters which have not yet been observed in our previous isolations. Rubeelu, an N-cluster phage, has an interesting immunity region similar to that characterized in phage Butters. As in Butters, the attP site was determined to be within the repressor gene and putative attB sites were found in *M. smegmatis* tRNAs. The second *M. smegmatis* phage isolated, Exilir, was found to have a myoviridae morphology similar to C cluster phage. However, PCR analysis with cluster C-1 and C-2 primer sets were unsuccessful, suggesting a novel C-cluster or singleton phage. To further increase the likelihood of discovering novel phage, the alternative host *Mycobacterium phlei* was used, from which only 8 phage have been isolated. Frankie, an F1 subcluster phage, is one of two F cluster *M. phlei* phage. Other *M. phlei* phage are in the A3, A13, and B3 subclusters. These results suggest that *M. phlei* has been overlooked as a source for potential mycobacteriophage. To explore *M. phlei*'s potential, and to better understand host range dynamics, 29

mycobacteriophage from the SEA-PHAGES repository, and 12 mycobacteriophage isolated at IRSC, were assayed for their ability to infect both *M. smegmatis* and *M. phlei*. Several phage were able to cross-infect, and represent a broad range of subclusters (A2, A9, D1, G1, K2, K4, L3, M1, M3 and a singleton). These studies increase the diversity of isolated mycobacteriophage and provide important insights into the host range potential of *M. smegmatis* and *M. phlei*.

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James Madison University  
Harrisonburg VA

Corresponding Faculty Member: Steve Cresawn (cresawsg@jmu.edu)



Emma C Eisemann

## ***The Diversity of Phages Compared According to their Hosts***

Emma C Eisemann, Fadak M Alali, Shannon L Ashby, Ashley N Ballengee, Corinne E Bastable, Burton E J, Rachel M Halterman, Kunny Kao, Anna M Labrozzi, Jason Lee, Niki J Lockwood, Noelle B Luis, Lily Y Mantz, Kinsey L Miller, Monica Paneru, Ashleigh N Queen, Catherine E Shola, Andrew Smith, Elizabeth F Terry, Sabrina M Thorsen, Matthew Todd, Katrina M Truong, Elizabeth A Lucas, Louie L Wurch, Steven G Cresawn

TattModd is an AK cluster bacteriophage isolated from Harrisonburg, VA on an *Arthrobacter* host. The TattModd genome is 43,546bp in length and encodes 62 genes. The TattModd genome includes structural genes in the left arm, genes for DNA replication and nucleotide metabolism, and then several reverse genes near the right end of the genome. *Arthrobacter* was selected as a host over *Mycobacterium* because of a perceived higher likelihood of discovering a singleton phage. As this turned out not to be the case, we then chose to investigate the probability of finding substantially novel phages with each of the seven most commonly used hosts in the SEA-PHAGES program.

The SEA-PHAGES database houses the complete genomic sequences of 1,983 bacteriophages. The majority of these phages (69% of the total) were isolated on *Mycobacterium smegmatis*. Phages have also been isolated on *Arthrobacter* (7.6%), *Gordonia* (9.4%), *Microbacterium* (2.2%), *Propionibacterium* (3.2%), *Rhodococcus* (2.6%), and *Streptomyces* (5.6%). The phages of these seven hosts are largely genetically distinct from one another. Comparisons between the seven datasets reveal substantial differences in their genetic diversity. For instance, 21.2% of *Rhodococcus* phages are singletons, while just 0.44% of *Mycobacteriophages* are singletons. This difference may be partially attributable to the relatively greater sampling of *Mycobacteriophages* relative to *Rhodococcus* phages, however differences are also evident when comparing similarly sized phage populations. For instance, just 1.3% and 1.6% of *Arthrobacter* and *Propionibacterium* phages, respectively, are singletons. As another measure of phage diversity, we compared the total number and sizes of (sub)clusters containing phages of each of the seven aforementioned hosts. *Mycobacteriophages* belong to the greatest number of these (71), while *Propionibacterium* and *Rhodococcus* phages each assort into just 3 clusters (excluding singletons). However, *Mycobacterium* and *Propionibacterium* clusters tend to be large, averaging 17.8 and 15.8 phages each. Smaller clusters indicate greater genomic diversity, greater sampling diversity, or both. By this metric, *Microbacterium*, *Gordonia*, *Rhodococcus*, and *Streptomyces* phages are similarly highly

diverse, with cluster sizes averaging  $< 4.1$  each. *Arthrobacter* phage clusters are intermediate, with an average size of 10.1 phages. For those wishing to isolate substantially unique phages, *Microbacterium*, *Gordonia*, *Rhodococcus*, and *Streptomyces* are excellent hosts. *Rhodococcus* and *Propionibacterium*, although still poorly sampled, appear to be hosts to a less diverse phage pool. *Mycobacterium*, while a host for a richly diverse set of phages, has already been well sampled and further efforts to isolate phages on this host would benefit from altering experimental conditions.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #036

James Madison University  
Harrisonburg VA

Corresponding Faculty Member: Louise Temple (templelm@jmu.edu)



Alexandra Lovrinic

### ***Prolate Podo Heads Don't Phage Us***

**Alexandra Lovrinic**, Hannah Clark, Paige Coatney, Greyson Davis, Andy Huang, Letu Kassa, Sophie LeFew, Kevin Loftus, Bryce Looney, Eleni Martinez, Cali McDonough, Jackson McNeal, Danny Mingo, Anna Miscione, Tommy O'Neill, Rachel Persinger, Jennica Pesino, Ali Shah, Jacob Sortore, Matthew Todd, Emily Williams, Jessica Cornell, Madison Himmelright, Blake Sutterfield

JMU Viral Discovery students isolated 50 phages using *Bacillus thuringiensis* Kurstaki (BtK) as the host, and 12 genomes were sequenced. Five of these were shown by TEM to be podoviruses and 7 were myoviruses. Four of the podoviruses, KonjoTrouble, Juan, RadRaab, and VioletteMad, were analyzed in detail. These podoviruses have prolate heads measuring 8 x 15 nm with short tails of 10nm or less. Around the neck, we observed unique appendage like structures. The small genomes ranged from 23929-26054 basepairs, and the GC content matched that of the host around at ~30%. The number of predicted genes ranged from 34-45. Of those, we were able to assign a putative function for about 14 genes. These four viruses have a highly conserved region encoding structural proteins at one end of the genome and a less well conserved region encoding enzymes or hypothetical proteins at the other end, as viewed using Phamerator. Several genes for typical structural components could be identified, including major head and tail proteins, and the gene encoding the conserved neck appendage protein was also identified. There is an unusual non-coding region that we have noted in similar podoviruses isolated at JMU and other SEA-PHAGES schools. These non-coding regions of about 800 bases are located in the left end of the genomes amongst hypothetical genes. Our BtK podoviruses are similar to the well-studied *Bacillus subtilis* phage, Phi29. All members of this family have discrete genome ends to which terminal proteins are covalently bound in the phage head and serve to prime DNA replication. The terminal protein gene was located next to the gene for DNA polymerase near the left end of our genomes. The ends of the genome in this family also have small inverted repeats. We noted identical repeats in the four phage genomes, which are 21 bases long with one mismatch. Among the structural genes was a lytic transglycosylase protein, which was similar to the tail lysin protein crystallized from Phi29. The lytic activity is contained in a protein at the tip of the tail, and the function is to locally break down the peptidoglycan structure to allow DNA to be inserted into the bacteria without breaking down the entire wall. These four phage genomes have been submitted to GenBank.

## 9th Annual SEA-PHAGES Symposium Abstract

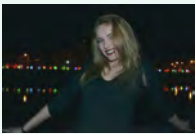
Poster #037

Johns Hopkins University  
Baltimore MD

Corresponding Faculty Member: Emily Fisher (efisher@jhu.edu)



Michaela Roskopf



Lindsay Young

### ***Duke\_13, Passionfruit, and Shiloh(s): Comparative analysis of annotated J-cluster genomes***

Julie Cui, Mackenzie Mills, **Michaela Roskopf**, **Lindsay Young**, Evan Qu, Wendy Xie, Alex Seo, Emily Fisher

Mycobacteriophages in the J-cluster have genomes almost twice the size of the typical myoviridae genome. While the average genome length for phages is around 60,798 base pairs, the J cluster has an average of 110,948 base pairs.

Duke\_13 is a J-cluster phage isolated in 2012 as part of the PHIRE program. Duke\_13 has a typical J-cluster genome size with 111,970 base pairs making up 246 annotated genes. Ninety one genes (~37%) were conserved in all 35 other J-cluster genomes based on analysis in Phamerator. We present core and accessory J-cluster genome and Phams of J-cluster phages were mostly conserved in the E, L, O, X, and Y clusters. None of the J-cluster phams was found in all other clusters.

The annotation of an adopted PHIRE genome came about due to our submission of a genome sample containing two J-cluster genomes, Shiloh-1 and -2. We present work attempting to separate these two from one another and comparing it to Passionfruit, which was isolated from the same original direct isolation plate in fall 2016.

This work adds to our understanding of conservation among these surprisingly long siphoviridae mycobacteriophage genomes and offers guidance for future J-cluster annotation projects.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #038

Kansas State University  
Manhattan KS

Corresponding Faculty Member: Christopher Herren (cdherren@ksu.edu)



Mackenzie Thornton



Marcy Anderson

### ***Flint Hills Phages - Analysis of Lysogeny in L2, A2, A3 and Other Uncharacterized Mycobacteriophages***

**Mackenzie Thornton, Marcy Anderson, Maggie Hill, Madeline Barger, Cheyenne Brunkow, Bailey Bye, Anna Burnett, Marlene Campos Guerrero, J. Alejo Carbajal, Jr., Adeline Chang, Christopher Clarkson, Amanda Curl, Samuel Douglas, Melissa Feuerborn, Jessica Garcia, Taylee Helms, Braden Johnson, Sydney Klein, Victoria Marsters, Ana Martinez, Matthew McCallister, Salvador Mosqueda, Destiny Parkhurst, Thomas Rafter, Jacob Richards, Jennifer Smith, Jazmine Snow, Stacy Vars, Kasey Wagner, Paige Wiebe, Thomas Williams, Coraima Yanez, Molly Zych, Martha Smith Caldas, Christopher Herren**

For 2 years, Kansas State University has been isolating mycobacteriophages from enriched soil samples in *Mycobacterium smegmatis* strain mc<sup>2</sup>155. This year's project also included analyses for lysogeny. Genome annotation can yield critical insights into potentially temperate phages. Finemlucis, the largest L2 subcluster phage found to date, ACFishhook, the smallest A3 subcluster phage found to date, and DBQu4n, an A2 subcluster phage were annotated this year and gave clues to potential lysogeny. Genome annotation of DBQu4n indicated the presence of an immunity repressor gene, integrase gene, excise gene and numerous stoperator binding sites in the genome required for binding of the immunity repressor and establishment of lysogeny. All of these genes are indicators of the common actinobacteriophage immunity system found in other A2 phages, such as mycobacteriophage L5. Annotation of the Finemlucis genome has shown the presence of an immunity repressor gene different from that of DBQu4n/L5, integrase, and excise. Interestingly,

the Finemlucis genome has no stoperator binding site sequences, but does have a gene coding for the CRO protein. The CRO (control of repressor's operator) protein is one of two proteins involved in a second mechanism for lysogeny like that seen in Lambda, P22 and 434 phages. Analysis of ACFishhook's genome indicates that it lacks both stoperator binding site sequences and the gene for the CRO protein, but it does code for integrase, excise and an immunity repressor protein. Adding to the bioinformatics analysis for potential temperate phages, all mycobacteriophages isolated thus far at K-State were screened in the lab to isolate functionally temperate phages. Stable lysogenic strains of *Mycobacterium smegmatis* strain mc<sup>2</sup>155 have been isolated that are infected with DBQu4n, Finemlucis, ACFishhook and several other Flint Hills phages that have not been characterized beyond electron microscopy. Subsequent phage release from each lysogenic strain has been demonstrated and all lysogens were further characterized for insensitivity to superinfection by the other Flint Hills phages.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #039

La Salle University  
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Corresponding Faculty Member: Nancy Jones (jones@lasalle.edu)



Michael Mercanti



Nicholas A Dingler

### ***Mycobacteriophages Chance64 and Ading: Siphoviridae vs Myoviridae***

**Michael Mercanti, Nicholas A Dingler, Cristian O Artilles-Hunter, Nathan Way, Liam Boger, Zachary Bolger, Bianca Brzezicki, Matthew Carey, Jennifer Chau, Isiah Deas, Payton Flores, Daniel Jonsson, Scott Kauffman, Madi Kuch, Phuc Luong, James Marr, Kevin McGowan, Jonae McKinney, Derrick Pinckney Jr, Victor Rotich, Zachary Schwartz, Brett Simon, Vincent Smith, Cian Sullivan, Malaika Wanjihia, Emily Wolbert, Luis F Almonte, Xiomaraliz Baez-Marengo, Fabian A Bergman, Jason E Chiara, Robyn I Crawley, Bryanna R Davis, Jordan Hagerty, Brianna Jankauskas, Terry Li, Hannah M McGary, Ryan W Nork, Christian Pemberton, Randy Rivera-Caro, Marisa B Sanchez, Katie M Stec, Cindy A Ung, Anthony Washington, Molly Wible, Tanya L Riddick, Marsha W Timmerman, Nancy L Jones**

In August of 2016, two Mycobacteriophages were discovered on the campus of La Salle University in Philadelphia, PA. On a temperate 70 degree day Chance64 was discovered on the surface of an outdoor ashtray by Michael Mercanti and Ading was discovered by Nicholas Dingler in the soil, six inches below the surface, surrounded by dying plants. Each of the samples was enriched using the host Mycobacterium smegmatis mc<sup>2</sup>155. Through many serial dilutions, web plates were acquired and plaques were measured at 1mm for Chance64 and 7.5 mm for Ading. Both phages produced clear plaques, meaning both were lytic. High titer lysates were harvested from the web plates and the lysates were used for obtaining a photomicrograph and also for DNA extraction. The photomicrograph of Chance64 showed it had a long tail and was a Siphoviridae. We were not able to get a good picture of Ading. Owing to the quality of the DNA extracted both Chance64 and Ading were sent to the University of Pittsburgh for sequencing. That is where the similarities came to an end. DNA sequencing confirmed that Chance64 is a Siphoviridae and that Ading as a C1 phage is a Myoviridae (having a short tail). Chance64 consists of 41,903 base pairs and has a GC content of

66.6%, while Ading consists of 157,399 base pairs and a GC content of 64.6%. Chance64 belongs to the G cluster and G1 subcluster and Ading belongs to the C cluster and C1 subcluster. Chance64 is made up of 62 total genes and 15 of those genes (24%) are dedicated to the construction of the tail, while Ading consists of 240 genes and 12 of those (5%) are dedicated to the construction of the tail. We hypothesized that since Ading was a Myoviridae and had a short tail that it needs more genes in other areas to strengthen the phage as a whole to compensate for the lack of a long tail. A quick look at Phamerator.org showed us that not all Myoviridae phages have long genomes. In addition, the number of lysin proteins is very small for both phages. Chance64 has two lysin genes and Ading has one. Ading also has a LysM, a protein that attaches to peptidoglycan using specific protein domains; this is the only LysM gene found between both Ading and Chance64. We examined the synteny by a pair-wise comparison of the tape measure protein and concluded that the protein was very different in these two phages. We will present the findings of phylogenetic comparisons of our phages and discuss our conclusions about their common ancestry.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #040

La Sierra University  
Riverside CA

Corresponding Faculty Member: Arturo Diaz (adiaz@lasierra.edu)



Nancy Kalaj



Casey Jang

### ***Crouching prolate, Hidden icosahedron: JangDynasty ends the corndog era***

**Nancy Kalaj, Casey Jang,** Brian Hwang, Shaquille Fyfe, Lorelei Hughes, Katarina Brankov, Thomas Pak, Jennifer Gallao, John Kim, Jason Tedjakusnadi, Emily Poon, Cecilia Saucedo, Connie Yang, Dong Yoon Han, Nicholas Fernandez, Truc Nguyen, Arun Muthiah, Natasha Dean, Nathaniel Sutter, Arturo Diaz

Sixteen Mycobacteriophages that infect *Mycobacterium smegmatis* mc2 155 were isolated from environmental samples and analyzed using SEA-PHAGES techniques at La Sierra University. Eleven of the phages were found via direct isolation while five were found via enriched isolation. The phages JangDynasty, Roliet, and Shaqnato were selected for Illumina sequencing and assigned to clusters O, B (B1), and C (C1) respectively. The full nucleotide sequence of JangDynasty was then further analyzed using DNA Master and related bioinformatic tools, which revealed a more detailed genetic profile of the phage. Mycobacteriophage JangDynasty is 70883 bp long with a 65.4% GC content and, according to the conjugate analysis of Glimmer and GeneMark, it contains 124 ORFs (67 of which are reverse transcribed) but no tRNAs, which is also the case for the other phages belonging to cluster O. The possible functions (not listed on NCBI Blast, PhagesDB Blast, or Phamerator) of two ORFs present in all Cluster O phages were determined based on HHPred evidence. Specifically, gene 98 was found to be highly similar to the ip1 gene (HHPred probability: 95.4%, e-value: 0.035), whose protein product is used by phage T4 to protect its DNA from the enzymatic activity of the two-gene-encoded *gmrS/gmrD* type IV dependent endonuclease discovered in pathogenic *Escherichia coli* CT596 (Rifat, Dalin, et al., 2008). Gene 90 was also found to have a 92.5% probability alignment to the CRISPR-associated protein Cse2 on HHPred. This protein was originally discovered in *E. coli* and seems to be most prevalent in bacteria. It may play a role in crRNA targeting of invading dsDNA and help stabilize the R-loop structures (Liu, Yuan, & Yuan, Journal of Structural Biology 2015) (Hayes, Xiao, et al., 2016). In addition, Mycobacteriophage JangDynasty displays a very evident structural difference with all phages belonging to cluster O. While cluster

O phages have unusual prolate capsids, JangDynasty has an icosahedral capsid, which is the prevalent shape among mycobacteriophages. Since no specific gene products determining the length of the capsid and thus the abnormal shape of prolate heads have been discovered studying cluster O phages (Cresawn et al., 2015), future genomic and proteomic analyses of JangDynasty could help identify the source of this peculiarity.

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Emily Benson



Broder Rachael

## ***Structural Characterization of Lysin A- and Holin-Coding Genes in the Mycobacterium Phage LittleLaf***

Emily Benson, Broder Rachael

After the isolation and sequencing of *Mycobacterium* phage LittleLaf, various bioinformatic tools were utilized in the annotation of its genome; these tools included the annotation software DNA Master, the gene prediction software GeneMark, the Starterator and Phamerator reports, Shine-Dalgarno values, BLAST, InterPro, and HHPred. The LittleLaf genome is 64834 base pairs long with a guanine-cytosine content of 63.4 percent. The genome contains 111 open reading frames. LittleLaf is classified as a Siphoviridae, a family of double-stranded DNA viruses, and is one of only seven phages to be assigned to Cluster S. LittleLaf's genes 53 and 56, which putatively code for Lysin A and Holin, respectively, have been selected for further study. Lysin, widely known as endolysin, internally hydrolyzes the cell wall of the bacterial host, where it targets the peptidoglycan in order to interfere with cell wall structural integrity. By breaking down the cell wall, newly assembled viruses can be released at the end of the lytic cycle. Holin is a group of small proteins that accumulate in the host's cell membrane, where they form holes that will allow lysins to access the host's cell wall. Ultimately, without Holin, Lysin would not be able to access and breakdown the cell wall and release newly formed virus progeny. Because of the location proximity and the codependency of these two genes, they are referred to as the lytic cassette. Previous research has shown that lysins, and holins by association, are effective in the treatment of infections induced by Gram-positive bacteria in lab animals. It has also been discovered that coupling lysins with other disruptive proteins is an alternative to combat the more troublesome Grams negative bacteria. The presence of Lysins and Holins coding genes in LittleLaf's genome would enable the use of this phage in the development and implementation of alternative antibacterial therapies.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #042

Lehigh University  
Bethlehem PA

Corresponding Faculty Member: Vassie Ware (vcw0@lehigh.edu)



Lauren M Pyfer



Wascar A Ramirez

### ***Characterization of Mycobacteriophage Genomes and Investigation of N Cluster Immunity Mechanisms***

Lauren M Pyfer, Wascar A Ramirez, Francis Akoto, Alexa Alhout, Netta Cudkevich, Matthew S Grapel, Maaz Haleem, Maryam Khan, Jamie B Korenberg, Brooke P Lichak, Lauren N McKinley, Kourtney R Mendello, Julia R Reisner, Rachel H Swope, Matthew J Thoonkuzhy, Lauren A Vargas, Croidy A Veliz, Kevin D Zhang, Caitlyn M Zuilkoski, Dylan Z Faltine-Gonzalez, Catherine M Mageeney, Margaret A Kenna, Vassie C Ware

Lehigh's SEA-PHAGES program is a collaborative research enterprise for first year and advanced undergraduates who focus on isolating and characterizing Actinobacter phages to gain a better understanding of phage genome structure, gene function, and phage biology in general. In addition to uncovering new phages for comparative genome analysis, our program focuses on novel phage genes that lack family members in other mycobacteriophages (called orphans) to understand their role in phage lytic or lysogenic life cycles. We report on progress on several projects. I. We highlight genomic profiles of newly isolated Mycobacteriophages Mitti (K4) and N cluster phages Kevin1 and Nenae - the latter two phages identified following DOGEMS analyses. Conserved and divergent genomic features for these newly annotated phages with other K4 or N cluster groups will be presented. II. Of special interest to our group is the N cluster – a group of temperate phages characterized by relatively small genomes of average size 43,111bp and a highly variable region centrally positioned within the genome. We have focused on Mycobacteriophage Butters that contains 4 orphans in the variable region, and have investigated homo- and heteroimmunity patterns with a host of mycobacteriophages as well as putative roles of orphans *gp30* and *gp31*. Recently, a novel mechanism of prophage-mediated immunity was uncovered for N cluster lysogens which provides defense against attack by variable groups of heterotypic mycobacteriophages and appears dependent on genes in the variable region of N cluster genomes (Dedrick et al., 2017). Further investigation of defense mechanisms includes an approach

to isolate heterotypic mutant phages (e.g., PurpleHaze, Island3) that overcome the Butters prophage-mediated defense system. Progress in characterizing “defense escape mutants” will be discussed. III. Isolation of N cluster phages Kevin1 and Nенаe allows for further exploration of prophage-mediated defense systems. The Kevin1 genome is more similar to Butters (e.g., both have lysis A and B genes) while the Nенаe and Redi genomes are highly conserved and contain a single lysis gene. A major difference between Kevin1 and Butters is the presence of Kevin1 orpham *gp30*, predicted to function as an AAA ATPase. Whether or not this predicted AAA ATPase is part of the defense mechanism against heterotypic viral attack is unknown. BRED experiments are in progress to delete Kevin1 *gp30* as a first step to determine its possible role in the phage life cycle or in defense against viral attack. Further, variable region differences in Butters, Kevin1, and Nенаe genomes predict different patterns of defense against viral attack for each lysogen. Immunity experiments confirm this prediction. Collectively, these experiments will provide further insights into the role of novel genes in the variable region of N cluster genomes in specifying immunity patterns that protect bacterial hosts from viral attack.

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Lehigh University  
Bethlehem PA

Corresponding Faculty Member: Vassie Ware (vcw0@lehigh.edu)



Vassie C Ware

## **Identification of a Role for Mycobacteriophage Butters-encoded Proteins in a Host Defense Mechanism Against Viral Attack**

Catherine M Mageeney, Marta Dies Miracle, Javier Buceta, Vassie C Ware

Our interest in Mycobacteriophage Butters (an N cluster phage) originally stemmed from the discovery that this phage has one of the smallest known annotated genomes (41,491bp with 66 genes, including 4 genes [*gp30*, *gp31*, *gp33*, *gp34*] with no known mycobacteriophage family members [called orphans]). Genome comparisons with other N cluster phages show extensive nucleotide conservation in structural assembly genes in the left arm and considerable divergence in nucleotide sequence and gene content in the central region of the genome, referred to as the “variable region”. Considering the large variation in genome size among mycobacteriophages (41,077-164,602bp), Butters was not only an ideal candidate to explore questions about minimum size requirements for genome packaging, but it was also ideal to determine if its orphans (located in the variable region) are required for a productive lytic or lysogenic life cycle. Recently, a novel mechanism of prophage-mediated immunity against viral attack has been reported for N cluster mycobacteriophages (Dedrick *et al.*, 2017). This novel mechanism appears dependent upon genes within the variable region and enables N cluster lysogens to defend against attack from a diverse group of heterotypic mycobacteriophages. We have explored the role of Butters *gp30* and *gp31* (both co-expressed in the Butters lysogen) and report on their contributions to the Butters defense system against viral attack. Initially, computational analyses predict that Butters *gp31* encodes a 4-pass transmembrane protein and that *gp30* encodes a protein with no membrane domains. We tested the computational hypothesis by expressing a C terminal tetracysteine-tagged GP31 and control ORFs within *E. coli* and imaged tagged proteins using fluorescence microscopy. Data show that Butters GP31 resides within the *E. coli* membrane coincident with a membrane marker. GP30 is cytoplasmic within *E. coli* with no membrane overlap. When tagged GP30 is co-expressed with untagged GP31, GP30 appears to be sequestered at the membrane, showing that the presence of GP31 alters GP30 localization. We hypothesize that GP30 and GP31 may therefore interact at the membrane. Immunity experiments using *Mycobacterium smegmatis* strains that express *gp30* or *gp31* alone, or both genes, show that defense comparable to that mounted in the Butters lysogen can be recapitulated only in the *M. smegmatis* strain that expresses *gp30* alone. Taking the imaging and immunity data together, we propose that GP30 is instrumental in conferring defense against specific viral attack only in its cytoplasmic state. What



factors regulate a proposed release of GP30 from the membrane (mediated through GP31) are unknown, but other genes (expressed in the lysogen) from the variable region may contribute to regulating this phenomenon. A model for Butters defense against viral attack involving *gp30* and *gp31* will be presented.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #044

LeTourneau University  
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Corresponding Faculty Member: Greg Frederick (GregFrederick@letu.edu)



Connor S DeJager



Megan S Perez

### ***Comparative Analysis of Mycobacteriophage Minor Tail Protein Carbohydrate Binding Module Polymorphism and Host Range***

Connor S DeJager, Megan S Perez, Nicholas R Leonard, Frederick N Baliraine, Gregory D Frederick

Mycobacteriophages are divided into clusters, based on overall nucleotide sequence homology. Within a given cluster, significant sequence variation is uncommon. Conversely, areas of strong nucleotide sequence homology between phages from different clusters are atypical. An intriguing example of cross-cluster homology was found in the cluster N Mycobacteriophage Andies through BLASTn analysis against the PhagesDB phage collection. Gene 20 of phage Andies and gene 21 of the cluster P phage Fishburne share a small segment of homology. In both, these genes code for a minor tail protein.

Further investigation indicated that this small shared segment of homology is likely the result of genetic recombination between different phages. Phamerator analysis revealed the region to be intragenic, within the genes, as opposed to encompassing the entire gene or larger regions of the genome. This is analogous to dissecting an automobile, inserting a slice from another model of automobile, fusing these together and producing a novel functional automobile. It is implausible that the parts, or in this case the protein domains or motifs, would align correctly to produce a functional product. The recombinant minor tail protein gene 20 of phage Andies is functional; otherwise Andies would be unable to infect host cells. HHPred analysis showed this homologous region to match the carbohydrate binding module (CBM) of hyaluronate lyase with 98.7% probability. Interestingly, sequence homology to this specific CBM was found in the minor tail proteins of

phages from a broad range of clusters. However, the specific amino acid sequence of the CBMs differs greatly between clusters.

This suggests that malleability in the CBMs allows for conservative amino acid substitution, thereby retaining function despite alterations in CBM primary structure. The consistent appearance of this specific CBM across phage clusters may warrant its classification as a conserved domain. These CBMs presumably aid phages during attachment to their bacterial hosts. Given that each cluster has its own unique CBM amino acid sequence, it was intriguing to investigate whether differences in the CBM amino acid sequences affect the phage's host range. To explore this, RasMol was used to produce 2D images of conformational models for the CBMs of various phages, and Espresso was used to determine structural homology. These bioinformatic softwares facilitated both sequence-based and structure-based comparisons of CBMs across phages from different clusters. The minor tail protein CBMs of 22 phages from 10 clusters/subclusters were analyzed. Infectivity data from 10 host strains considered together with CBM analysis showed that differences in the carbohydrate binding module does influence phage host range.



# SEA PHAGES

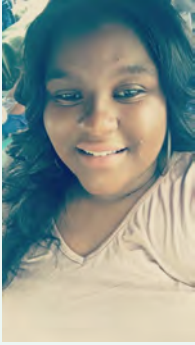
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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #045

Lincoln University  
Lincoln University PA

Corresponding Faculty Member: David Royer (royer@lincoln.edu)



Shekinah L Vann



Joy Yaki

### ***Kasen3, a newly annotated G1 subcluster mycobacteriophage***

Shekinah L Vann, Joy Yaki, Deanee N Love, Dashawn N Asegbola, David F Royer

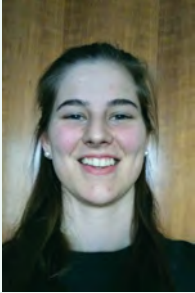
Kasen3, a G1 subcluster bacteriophage, was isolated from a soil sample collected on the campus of Lincoln University as part of a Phage Hunters class offered during the 2016-17 academic year. Kasen3 is similar to other G1 subcluster phages in genome length, GC content and number of genes. The greatest similarity is to G1 phages Angel (western PA), Halo (Pittsburgh), Taheera (South Africa) and Sneeze (Costa Rica). It is a member of the Siphoviridae with a characteristic long tail. The annotation revealed 61 genes, of which 32 were assigned a putative function based on comparisons with other G1 mycobacteriophages using BLASTP assignments from both phagesdb.org and GenBank. Of the 32 genes assigned a function, 18 coded for structural proteins and 14 coded for functional proteins. A programmed translational frameshift involving genes 14 and 15, tail assembly chaperones, was identified.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #046

Marywood University  
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Alyssa Tirella



Marlee Holleran

### ***'Phaging' the World: Digging for a Way to Combat Antibiotic Resistance***

Abigail Hein, Alexandra Graziano, **Alyssa Tirella**, Ama Gajanayake, Amanda Lisi, Angela Ngo, Angelo Brutico, Camille Kotcho, Christina Kazanas, Christopher Cariffe, Christopher McCann, Courtney Paulus, Dekonti Sayeh, Emily Medwid, Emily Onyshczak, Emma Moser, Hannah Goble, John Grossi, John Shebby, Joshua D'Agostino, Julianna Turnbach, Justine Chanthachackvong, Kayli Black, Logan Brighton, Logan Shook, Mariah Casey, **Marlee Holleran**, Noah Karasik-Tiewski, Patricia Lee, Paul Matarazzo, Siena Senn, Toni Shipman, Trevor Flynn

The World Health Organization in 2014 estimated that by 2020 most, if not all, antibiotics currently prescribed will no longer be effective, therefore, a new method of treatment must be created and prepared before this issue arises. Bacteriophages, viruses that kill bacteria, may be used as an important aspect of these new treatments. The Science Education Alliance - Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) is a national project that encourages undergraduates to research mycobacteriophages, with the goal of using them as a treatment or cure for bacterial infections, such as Tuberculosis, while also exposing students to the process of field research. The specific bacteria used in the lab at Marywood University was *Mycobacterium smegmatis*, also known as *Smeg*, which is closely related to *Mycobacterium tuberculosis*. During a two-semester course consisting of a wet lab and a bioinformatics lab, student researchers collected soil samples to extract phage and characterize it. Students performed enriched isolation, plaque assays, spot

assays, serial dilutions, DNA isolations, and restriction digests. Within the SEA-PHAGES program at Marywood University, two phages, Faze9 and Roy17, were discovered, amplified, and sequenced. Faze9 belongs to the B2 subcluster and contains 67,503 base pairs initially predicted as 91 genes that were trimmed to 88 genes. Roy17 is a B1 subcluster with 104 initially predicted genes and 68,056 base pairs, of which 98 genes are found in the finished annotation. Per the TEM performed at Drexel University, Roy17 has a 162.5nm tail and a 31.25nm head, while Faze9 has a 373 nm tail and a 83 nm head. Faze9 has a 68.9% GC content, while Roy17 has a GC content of 66.6%.

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Massey University  
Auckland Campus

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### ***Discovery, characterization and LIGHT microscopy of two novel Pseudomonas phages that make nucleoid-like compartments in their bacterial host: Phabio and Noxifer.***

Joanna Wojtus, Jess Fitch, Eli Christian, Jaclyn Medland, Theresa Gracie, James Conway\*, Eric Altermann, Heather Hendrickson

\* University of Pittsburgh, Pittsburgh PA

*Pseudomonas* is a lineage of bacteria that includes the aetiological agent responsible for cystic fibrosis and a number of beneficial plant associated microbes. One of the latter, *Pseudomonas fluorescens* SBW25 is also commonly used to study rapid niche adaptation in experimental evolution. We used *P. fluorescens* SBW25 as our target organism for our first phage hunt in New Zealand in 2013. We discovered and sequenced two new Myoviridae phages, classified as Jumbophages (genomes >200 kb) Noxifer and Phabio. Genome sequencing revealed that our Jumbophages have a number of interesting genetic information processing –homologs including T4-like DNA polymerase, DNA ligase, RNA polymerase subunits and DnaB helicase. Phamerator analysis reveals that these homologs are common in the *Pseudomonas* Jumbophages and that long term synteny in the midst of nucleotide divergence appears to be the norm in this group.

Noxifer and Phabio have not been found to infect other strains of *Pseudomonas* species tested (n=5) and are likely to have a limited host range. Each has a relatively small burst size, on the order of ~20 phages are produced in a round of infection. In addition, both of these Jumbophages are capable of transduction and can move host DNA between cells.

Recent work on a member of this Jumbophage group, 201phi2-1 demonstrated the ability to form nucleoid like compartments in which DNA replication and transcription take place but translation and capsid formation take place externally. Phabio and Noxifer are also able to produce compartments in *P. fluorescens* SBW25 during infection and these cause visible perturbations of the cells under light microscopy (Phase and DIC). We have used fluorescent microscopy to visualize the host genome during phage infection and found that host DNA appears to persist in our cells during viral infection. The persistence of this fluorescently labeled host DNA may help us to elucidate the mechanism that makes transduction common in Jumbophages.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #048

Merrimack College  
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Jessica Izurieta



Justine Levesque

### ***Cornucopia and Piper2020: A Clear Choice***

Jessica Izurieta, Justine Levesque, Charlotte Berkes, Janine M LeBlanc-Straceski, Christina MacLaren

Cornucopia and Piper 2020 are two cluster F1 Mycobacterium smegmatis phage with distinctly different plaque morphologies. Cornucopia is a temperate phage that produces cloudy plaques, while Piper2020 appears to be lytic, producing only clear plaques. These phenotypes correlate with the presence of an integrase gene in Cornucopia and the absence of one in Piper2020. Cornucopia was discovered in the soil collected from an active vermiculture compost bin, a few inches under the surface. Piper2020 came from the soil of a tomato plant that was watered daily throughout the summer. Both of these phages have between 95-97% of their genes transcribed in the forward direction. Blast comparison between these two phages showed a >90% sequence homology throughout the first 30,000 bp and the last 3000 bp of their genomes. The region in between had much lower homology, many different genes and some similar genes arranged in different patterns. The Cornucopia integrase gene is 1128 bp and has a >95% homology to five other cluster F1 phage integrases throughout the entire gene. However, in many other F1 phages, the 5' portion of the Cornucopia integrase gene is missing. MooMoo, a singleton, with a similar halo plaque morphology to Cornucopia, contains a similar integrase that includes this 5' region. Nucleotide tblastx of this 5' region of Cornucopia's integrase gene showed that this is a common motif found in many phage genomes. Further analysis will determine whether this domain is found in other integrases, or proteins with other functions.



## 9th Annual SEA-PHAGES Symposium Abstract

Poster #049

Miami University  
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Sydney E Arlis



Allison M Biedenharn

### ***Increasing Bacteriophage Diversity Through the Use of Variant Culture Conditions and DOGEMS***

Sydney E Arlis, Allison M Biedenharn, Emily R Konrad, Cole J Goodrich, Steven M Madias, Jon E Abell, Anastasia C Babcock, Amanda C Burgess, Luke R Carrafiello, Callen R Conroy, Cortney F Mitchem, Paolo Palacios, Julie M Donna, Steph M Smith, Hailey E Riggs, Mariah S Squire, Luis A Actis, Rebecca S Balish, Mitchell F Balish

The output of the SEA-PHAGES program over the years has uncovered great diversity among cultured bacteriophages that infect *Mycobacterium smegmatis*. Genome sequence analysis of these bacteriophages reveals that most fall into one of 26 phylogenetic clusters. However, for a variety of reasons, some of these clusters are highly over-represented in the Actinobacteriophage Database, whereas representatives of other clusters are much less frequently encountered by SEA-PHAGES students. The development and application of techniques aimed at increasing phylogenetic diversity of bacteriophages during both the culturing and sequencing phases is likely to reduce this skew. Toward this end, the students in Miami University's MBI 223 and 224 courses in 2016-2017 isolated 19 bacteriophages capable of infecting this bacterium from soil samples and cultured them using standard techniques with some modifications aimed at increasing diversity. Culturing at 30°C by some students contributed to the purification of two bacteriophages from cluster C. Using the recently developed DNA sequencing protocol, Deconvolution of Genomes after En Masse Sequencing (DOGEMS), the SEA-PHAGES team and we selected three rarely encountered sequences from phylogenetic subclusters D1, N, and A12 from a mixture of the genomic DNA from 18 of the bacteriophages for analysis. Through polymerase chain reaction analysis of genomic DNA from individual bacteriophages, the D1 and N

entries were identified as Erk16 and Silvafighter, both isolated on the campus of Miami University, whereas the A12 bacteriophage, which was missing some sequence information from the right end of the genome, was not unambiguously identified from the pool of bacteriophages discovered by the students. Annotation of the Erk16 revealed low diversity of gene content as compared with other subcluster D1 bacteriophages. The Silvafighter annotation highlighted somewhat more variant gene content within the N cluster. The incomplete subcluster A12 bacteriophage was the most distinct in terms of gene content. Despite the failure to identify the A12 bacteriophage, we affirm that together with modification of culture conditions, DOGEMS is a very effective means of selecting rare bacteriophages from a population.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #050

Montana Tech of the University of Montana  
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Macy K Ricketts



Chloe E Bayless

### ***Structural differences between tape measure proteins of unique Actinobacteriophage clusters and two novel Montana Mycobacteriophages***

Macy K Ricketts, Chloe E Bayless, Margeaux S Black, Marisa L Pedulla

Within a bacteriophage genome, the tape measure protein (TMP) dictates the length of a bacteriophage tail. In addition, this protein may function in signaling to activate non-replicating bacterial host cells. The gene that encodes for TMP is typically large in size and thus can be identified in a bacteriophage gene map when taking into consideration the size and proximity to specific programmed ribosomal frameshifts in upstream protein coding genes (Pedulla et al., 2003). The function of TMP was determined by a team of researchers in 1984 (Katsura and Hendrix). The team found that upon removing segments of the gene, the bacteriophage exhibited a proportionally smaller tail, suggesting a direct correspondence between TMP gene length and the length of the phage tail. While subsequent studies have supported these data, several bacteriophages have been found to contain portions of the gene that do not contribute to tail length (Pedulla et al., 2003).

The objective of this study was to compare structural differences in TMPs belonging to different phage clusters. We first annotated the genomes of two novel Mycobacteriophages, MrYolo and Froghopper, and identified the gene encoding tape measure protein in each genome. The putative secondary and tertiary structures of these TMPs were determined using prediction software (PredictProtein, 2017). From the Actinobacteriophage Database we then selected TMP amino acid sequences of 4 phages each from clusters A,

B, K, and F, (Russell & Hatfull, 2016), and software was used to predict the secondary and tertiary structures of each TMP selected. Finally, we ran ClustalW alignments, and generated phylogenetic trees showing the relationship among TMPs of the selected phages (Bioinformatics Center, 2017). The results of this study showed that TMPs within clusters are more closely related and structurally similar than those of different clusters.

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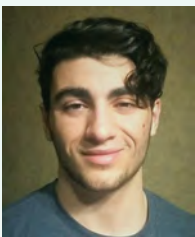


Montclair State University  
Montclair NJ

Corresponding Faculty Member: Sandra Adams (adamssa@mail.montclair.edu)



Gerard Nasser



Adam Zadeh

## ***Isolation and Characterization of Novel Mycobacterium smegmatis Bacteriophage from New Jersey Soil Over the Past Six Years***

Gerard Nasser, Adam Zadeh

In 2016, 35 lytic phages that infect *M. smegmatis* were successfully isolated and further characterized through plaque morphology and scanning electron microscopy. Genome sequencing of two of these phages (MoneyMay and Tarynearal) revealed both were novel, previously undescribed phage. MoneyMay is a subcluster A3 phage with a putative 91 genes (including one tRNA gene). Tarynearal is a subcluster A5 phage with a putative 93 genes (including three tRNA genes). Students from the MSU Howard Hughes SEA-PHAGES Genomics course have isolated 94 bacteriophages since 2011. Of these phage, 10 have been sequenced, five have been published in GenBank, three are in review for deposition in GenBank, and two are currently being annotated. The MSU SEA-PHAGES team has also been developing cluster and subcluster-specific PCR primers. Current methods of identifying a phage's cluster include morphological analysis using electron scanning microscopy and whole genome sequencing. Microscopy does not always positively identify cluster (or sub-cluster) since some clusters are morphologically similar and genome sequencing is prohibitively expensive for large numbers of samples. An alternative approach is to develop a PCR-based technique using cluster-specific primers based on unique genes in each cluster. To identify candidate genes Phamerator was used to align each cluster of phages to find 4 pieces of data: (1) E-value and percent similarity between the phages in the cluster, (2) location of the candidate gene in relation to each phage in the cluster, (3) gene length, and (4) cluster specificity (whether or not the gene in question is in any other phage outside the cluster). From there, primers are to be made for each unique gene. To date, clusters A1, A7, and A10-18 have had 91 unique genes found, from which 33 candidate genes have been identified and work continues to identify candidate genes in the other clusters as well as test primers for each cluster.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #052

Morehouse College  
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Del-Vecie Brown



Brian Davis

### ***Annotation of Mycobacteriophage Nairb***

Aaron T Brown, **Del-Vecie Brown**, Philleon K Chambers, Albert T Clinton, **Brian Davis**, Kahlid H Fowlkes, Zaki S Gregory, Carlos M Hill, Joshua P Liggins, Joshua A McNeil, Kareem M Moore, Quadir J Phillips, Darnell A Taylor, Alexandra Peister, Lawrence S Blumer

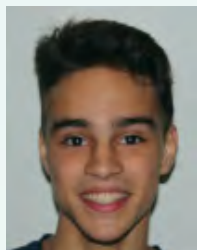
Mycobacteriophage Nairb was isolated from a soil sample in Atlanta, Georgia, using *Mycobacterium smegmatis* as the host. Nairb is a Cluster T phage that is 42,393bp long. The purpose of our research was to finalize the draft annotation of this phage by manually confirming potential genes and identifying gene functions. Utilizing the annotation program, DNA Master, and guided by heuristic GeneMark output we determined the most likely open reading frames to identify each gene in this genome. Starterator reports were used to help identify the most conserved starts in each pham, and BLASTp searches and HHPred searches were used to identify the function of more than half of the genes in this genome. In our completed annotation, there are a total of 59 genes in Nairb and no tRNA or tmRNA sequences. The functions of 35 of the 59 genes were identified. We were particularly interested in the similarities and differences between this phage and other four phages in Cluster T. Synteny is very clear in all these phages in the first half of each genome. The two previously annotated Cluster T phages (Bernal13 and Ron RayGun) and Nairb have a programmed translational frame shift in the tail chaperone genes just upstream of the tape measure gene. The presence of integrase (Yt int), immunity repressor, and excise genes suggest that Nairb is capable of lysogeny. Comparisons between the Phamerator maps showed remarkable similarity in both nucleotide sequences and protein products early in each genome. Most of the differences between the five Cluster T phages is observed in the second half of the genomes.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #053

Mount Saint Mary College  
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Ishaan Brissette



Ryan Forster

### ***A Phage Tale: Isolation and Characterization of a novel G1 sub-cluster phage Octavious Rex***

Ishaan Brissette, Ryan Forster, Andrew Barberio, Andrew Clemente, Catrina Colombo, Matthew Conboy, Jacqueline Copeland, Erica Dominguez, Emily Gilligan, Molly Jensen, Pascal Kadamani, Carrie Massari, Jenna Olivett, Valerie Smith, Jared Whitford, Suparna Bhalla, Evan Merkhofer

Mycobacteriophage Octavious Rex was one of sixteen novel phages isolated by undergraduates at Mount Saint Mary College during the fall 2016 semester. Identified as a result of direct isolation, Octavious Rex was purified to homogeneity after three rounds of full plate plaque assays. Transmission Electron Microscopy (TEM) revealed that Octavious Rex was a member of the siphoviridae family. DNA isolated from the high titer lysates was submitted to the University of Pittsburgh for Illumina sequencing. Sequencing results determined the genome length of Octavious Rex to be 41880 base pairs with a GC content of 66.6%. This phage was classified as a G cluster bacteriophage. Furthermore, it was also determined to be a member of the small sub-cluster G1. As part of the spring 2017 semester, undergraduates successfully annotated the viral genome of Octavious Rex. Bioinformatic programs including DNA Master, Gene Mark, Phamerator and Starterator were used to achieve this goal. Where applicable, gene functions were assigned using HHpred and NCBI BLASTX programs. Previously identified G1 phages have been characterized as lysogens. Annotation of Octavious Rex revealed the presence of immunity repressor and integrase genes, previously found to be associated with lysogeny. Though identified as a lytic phage, we are currently investigating the potential lysogenic life cycle of Octavious Rex. Previous studies by the Turner lab have shown changes in thermotolerance of phages upon exposure to non-ideal temperatures. We are currently investigating if similar changes occur in Octavious Rex upon exposure to heat shock. These studies will advance the knowledge and understanding of G1 subcluster mycobacteriophages.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #054

Nebraska Wesleyan University  
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Dayton Dolincheck



Johan Vizoso

### ***Comparison of Bacteriophage OlinDD and RobsFeet to other isolated Microbacterium paraoxydans bacteriophages***

Dayton Dolincheck, Johan Vizoso

The SEA-PHAGES program allows undergraduates the opportunity to isolate novel bacteriophages from the environment. The DNA from each isolated bacteriophage is sequenced and the genome annotated using a variety of computer software and online tools. Bacteriophages OlinDD and RobsFeet were isolated from the host bacterium *Microbacterium paraoxydans* NWU1. These two bacteriophages were compared to one another. OlinDD is a Siphoviridae, classified in cluster ED, and has 114 putative genes. RobsFeet is a Myoviridae, classified in Cluster EC, and has 101 putative genes. Since *Microbacterium paraoxydans* NWU1 is a new host, information regarding the genome arrangement, genes obtained, in addition to determining commonalities between these two bacteriophages and other bacteriophages isolated from *Microbacterium paraoxydans* will be discussed.



## 9th Annual SEA-PHAGES Symposium Abstract

Poster #055

North Carolina A&T State University  
Greensboro NC

Corresponding Faculty Member: Roy Coomans (coomansr@ncat.edu)



Evan Pardue

### ***Aggie and Philonius, Two New Cluster N Mycobacteriophages from North Carolina***

**Evan Pardue, Nalani Brown,** Kedeja Adams, Abiola Adesoye, Ricki Bates, Amina Bradley, Rixon Campbell, Russell Covington, Jonathan Culler, Chelsea Daye, Lexis Deshazor-Burnett, Drevon Dobson, Donovan Dooley, Lauren Enoch, Taylor Evans, Adrinna Freeman, Synphane Gibbs, Tkeyah Harvey, Brittany Horne, Najawa Huntley, Shayla Jackson, Zachary Jackson, Antwynette King, Willie Lisane, Louisa MacCormack, Stephen Melvin, Zchelani Mulcare, Alexis Whidbee, Deja Wilson, Supriya Patwardhan, Cameron Hopkins-Harrington, Robert Newman, Roy Coomans

During the Fall of 2016 the two SEA-PHAGES classes at North Carolina Agricultural and Technical State University (NC A&T) isolated ten phages from environmental samples using *Mycobacterium smegmatis* mc<sup>2</sup>155 as the host organism. All ten were isolated from enrichment cultures using standard SEA-PHAGES protocols. DNA extracted from four of the ten phages was sequenced at Pittsburgh Bacteriophage Institute. All four phages proved to be novel. Two of the four sequenced phages, Aggie and Philonius, were found to be in Cluster N, a small cluster that contains 23 sequenced and verified members, representing only 1.2% of the total number of phages in the Actinobacteriophage database. Both phages were isolated from soil samples collected on the campus of NC A&T in Greensboro, North Carolina. Aggie and Philonius have genome lengths of 44,333 bp and 43,886 bp respectively; both have a GC content of 66.5%. These values are in line with the Cluster N averages of 66.2% GC content and 43,111 bp genome length. Genomes were annotated using DNA Master and PECAAN with input from Phamerator and Starterator. The genomes of Aggie and Philonius show a high degree of sequence homology with the exception of one region, less than 3,000 base pairs in length. Aragorn v.1.2.38 identified a tRNA gene for threonine in the genomes of both Aggie and Philonius, but this gene occurs in a forward reading frame within gene 35, a reverse gene that codes for an immunity repressor. Because of the overlap, the tRNA gene was excluded from the final annotation. During annotation, we investigated the usefulness of the protein structure prediction program I-TASSER as a tool to gain information about the structure and function of select gene products beyond that obtained from BLAST, HHpred, and the Conserved Domain Database.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #056

Northwestern College  
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Alison Schutt



Katie Inge

### ***Phage Hunting in the Midwest Prairie***

**Alison Schutt, Katie Inge,** Byron Noordewier, Sara S Tolsma, Samantha Bruinsma, Jeffrey Jeltema, Jose Muro, Peace Preston, Michaela Van Riesen, Alex Yoerger, Perkins Aiyegbeni, Myles Anderson, Madison Beaver, Christopher Borchers, Emily Geraets, Hailey Gestrich, Courtney Mithelman, Renju Pun, Kristina Sevcik, Sabrina Tarchione, Brenden Van Den Werff, Patrick Wagner

After ten years of phage hunting, SEA-PHAGES students have succeeded in discovering and annotating thousands of phages and their genomes. Most of these phages were discovered from samples collected in the Southern United States and on both the East and West coasts. Northwestern College is a SEA-PHAGES institution in Iowa and our students are contributing to the discovery of Mycobacteriophages in the Midwest. In our first year offering a SEA-PHAGES course, Northwestern College microbiology students discovered sixteen different Mycobacteriophages during the spring 2017 semester. Of fifteen soil samples, three soil samples yielded phages: nine by direct isolation and seven using the enrichment protocol. All sixteen phages grow on *Mycobacterium smegmatis* mc2155. Students in the fall 2017 genetics course at Northwestern College will annotate two of these Midwest Mycobacteriophages. While the microbiology students were busy discovering new phages, a group of seven honors research and directed study students annotated a phage adopted from phagesDB.org. ILeeKay, isolated in 2010 at Brigham Young University and sequenced in 2014, has a 51,017 base pair genome with a 64% GC content. It is a cluster A1 phage. We found that it contains 90 genes,

including genes for bacterial infection, genome replication, viral structure, and capsid assembly. ILeeKay's plaque morphology suggests that it is a lysogenic phage and we found a number of genes whose protein products are important for lysogeny including integrase and an immunity repressor. ILeeKay's genome does not include tRNA genes. The tail assembly chaperone gene contains a -1 programmed translational frameshift, similar to many other phages in the A1 subcluster. ILeeKay most closely resembles phages PhrostyMug, TheloniousMonk, and Zephyr, each of which are also cluster A1 phages and we propose that they are among ILeeKay's nearest evolutionary relatives.

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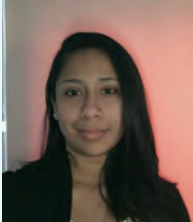


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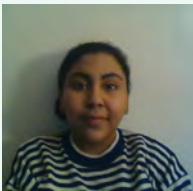
Poster #057

Nyack College  
Nyack NY

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Maridalia Lillis



Mariana P Moraes

### ***Exploring New Terrae-tories with Gordonia Phages***

Maridalia Lillis, Mariana P Moraes, Jellissa Garcia, Rachel Meiners, Jeremiah Sylvain, Peter J Park, Jacqueline M Washington

*Gordonia* sp. are aerobic Gram-positive bacilli that are related to *Mycobacteria* sp. as both are members of the phylum Actinobacteria. Compared to mycobacteriophages, little is known about other actinobacterial phages. Therefore, isolation and characterization of other types of actinobacterial phages will not only increase the diversity of bacteriophages but also give us further insight to phage biology and their evolution. During the 2016-17 academic year, Nyack College isolated bacteriophages using *Gordonia terrae* 3612 as a host and an isolation temperature of 28 degrees. Eight *Gordonia* phages were isolated, five of which were sequenced and identified as members of five different clusters (A15, CV, DN, DD, and DE). The DN cluster phages form a new cluster with six phages first isolated and identified in 2016 from sites in five states across the United States.

The genomes of the sequenced phages range in size from 49,965 bp (Fenry, CV) to 57,555 bp (Ashertheman, DE) with as few as 80 putative genes in Fenry and up to 108 genes in both Phistory (DN) and ShayRa (A15). Similar to other actinobacteria, *Gordonia terrae* has a high GC content of 67.8%. The isolated *Gordonia* phages range from a GC content of 62.1% (ShayRa) to 68.0% (Ashertheman). Interestingly, to date of the nine A15 phages, ShayRa is the smallest primarily due to a 1.3kb deletion in the region where the immunity repressor is typically found. Additional bioinformatic analysis of the phages will be presented.

To further investigate and characterize these phages, wet bench experiments were performed including SDS analysis, temperature sensitivity, determination of host range and immunity assays. SDS analysis of the structural genes revealed unusual results in that although the phages are members of different clusters, the

structural genes appear to be of identical sizes. All the phages were able to be grown at temperatures higher than the isolation temperature of 28 degrees, with the exception of Ashertheman (DE). Growth of Ashertheman at 37 degrees results in a several fold loss of viability. Interestingly, these temperature resistant mutants show similar growth profiles at both 28 and 37 degrees. Further experiments to understand the exact mechanism for the acquired temperature resistance are ongoing. To date, host range experiments show that these *Gordonia terrae* phages were unable to infect several strains including *Gordonia amarae*. As several of the phages are able to form lysogens, the results of immunity experiments will also be presented.

Lastly, DNA sequence analysis determined that one of our phages Chazimma was a mixture of DD and DE phages. Indiscernible by plaque morphology, we will present our work on techniques used to separate these phages which proved to be surprisingly challenging.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #058

The Ohio State University  
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Carina Bertolini



Alyssa Tyransky

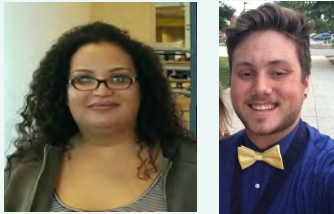
### ***Comparative Analysis of Cluster AV Arthrobacteriophages, Including Recent Isolates Adat, Gurgleferb, and Nellie***

Carina Bertolini, Alyssa Tyransky, Caroline Breitenberger, Sarah Ball, Charles Daniels

Since 2011, students at The Ohio State University have been isolating bacteriophage using the host *Mycobacterium smegmatis*. To date, they have isolated and purified over 150 mycobacteriophages and have had 20 genomes sequenced. This year, we decided to switch to *Arthrobacter* sp. as our host in order to contribute to the expanding knowledge of phage diversity. During fall semester 2016, students isolated 6 novel *Arthrobacter* phage including 3 belonging to cluster AV, which previously had only one known member (Jasmine). The genomes of these newly discovered phage (Adat, Gurgleferb, and Nellie) range in size from 45,426bp to 45,428bp, compared to Jasmine's 46,723bp genome. They all have 57 predicted open reading frames and an average GC content of 45.8%. 93% of their Phams are found only in cluster AV. Perhaps the most interesting feature is their morphology, as the AV cluster phages are the first podoviridae phage characterized by the SEA-PHAGES program to date. Genomic analysis failed to reveal a putative tape measure gene which is consistent with previously examined podoviridae phage. Additional bioinformatic analyses were performed to try and identify genes related to tail assembly.

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Delilah Hahn    Ryan Kilduff

## ***Host range project: Investigating if phage isolated from *Mycobacterium smegmatis* can infect pathogens of fish, amphibians and humans***

Delilah Hahn, Ryan Kilduff, Nazir Barekzi

Over 10,000 bacteriophages have been collected and submitted to the Actinobacteriophage Database. Among those, over 1000 have been sequenced through the SEA-PHAGES program. At ODU, we have been participating in the Host Range Project: SEA-PHAGES 2016-2017 to test whether a collection of phages can infect a host other than *Mycobacterium smegmatis* mc<sup>2</sup> 155. Host range studies are relevant in understanding the process of phage infection and the potential use of a single phage against multiple bacteria in phage therapy.

We received 30 mycobacteriophage lysates from the archives at University of Pittsburgh and used 5 phages isolated at ODU in this study. In order to determine if bacteriophage have more than one host, a collection of 35 phages have been investigated to determine if these phage have a broad host range. Originally, all of the phages were isolated with *Mycobacterium smegmatis* mc<sup>2</sup> 155.

The goal of this project was to determine if phage have a broad host range by testing each phage on different bacterial hosts within the Actinobacteria phylum. In total, thirty five phage and eight different bacterial hosts that are human and fish pathogens were investigated: *Mycobacterium smegmatis* mc<sup>2</sup> 155, *M. smegmatis* NSC 3240, *M. fortuitum* M5, *M. fortuitum* M6, *M. chelonae* M3, *M. chelonae* 324-818, *M. marinum* ATCC 927, and *M. marinum* M30-01. To test the broad host range infectivity, serial dilutions of each isolated bacteriophage were prepared, followed by spot tests of 3-5 ul of each dilution on a nutrient agar plate containing a bacterial lawn made from each strain.

Subsequently, the plates were incubated at 30°C and observed for the presence of plaques or zones of clearing. The results revealed that in addition to infecting *M. smegmatis* mc<sup>2</sup> 155, at least eleven phage were able to also infect *M. smegmatis* NSC 3240, at least two phage infected *M. fortuitum* M5, and one phage (BxB1) infected *M. fortuitum* M6. The titers of the phage and the efficiency of infection was calculated using the phage titers from *M. smegmatis* mc<sup>2</sup> 155 as the control. These findings indicate that some bacteriophage isolated on *M. smegmatis* mc<sup>2</sup> 155 can infect related species of bacteria and have a broader host range than originally thought. However, the range of hosts may be limited due to the genomic composition and evolution of each distinct phage.

# 9th Annual SEA-PHAGES Symposium Abstract

Ouachita Baptist University  
Arkadelphia AR

Corresponding Faculty Member: Nathan Reyna (reynan@obu.edu)

**Poster #060**



**Kayla L Haberman**



**Charles M Burnham**

## ***DOGEMS! It's RiverRat***

**Kayla L Haberman, Charles M Burnham, Brittany P Backus, Chelsey D Vermillion, Justin C McGee, Jonathan L Askins, Addison K Bostian, Kinnon S Dodson, Aitor Lopez Breton, Sam B Eddington, Harrison S Ballard, Noah S Nalley, Sarah K Vickers, Daniel N Games, Logan T Bond, Nathan S Reyna, Ruth C Plymale**

Students at Ouachita Baptist University (OBU) used DOGEMS (Deconvolution of Genomes after En Mass Sequencing) on a pooled phage sample to increase the chance of finding a novel phage. Isolated DNA, from ten *Mycobacterium smegmatis* phages, were combined and submitted for DOGEMS. Unique phage in the mixture will separate out as individuals during the sequenced contig assembly step. We recovered a single B4 phage from our mixed sample, as well as multiple A, F1, K3, and L2 phage. The next step was identifying this single B4 phage from among the ten phage in the pooled sample. Student groups studied sequenced B4 phage genomes and designed unique polymerase chain reaction (PCR) primers specific to the B4 subcluster. One group created primers for pham 1320, a unique gene only found in B4 phage. This primer set produced a 798 bp amplicon in a known B4 phage and RiverRat. No other phage in the pooled DOGEMS sample produced a PCR product. Other student groups, using unique primer sets, independently confirmed the single B4 phage in our DOGEMS sample as RiverRat. We will discuss the rationale used to design each group's primer set.



## 9th Annual SEA-PHAGES Symposium Abstract

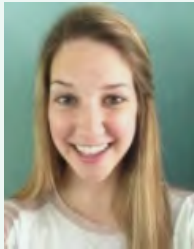
Poster #061

Purdue University  
West Lafayette IN

Corresponding Faculty Member: Kari Clase (kclase@purdue.edu)



Kate Carpenter



Francesca Whitt

### ***Comparative genomic analysis and genome annotation of mycobacteriophages AFIS and JewelBug***

Madalyn Alm, Kathryn Atherton, Sarah Bell, Diane Besich, Sharifah Binti Syed Omar, Jacob Bosler, Lizzie Canida, **Kate Carpenter**, Celine Chang, Yvonne Chen, Audrey Conrad, Larkin Cooper, Nick Fields, Mahima Grover, Margaret Hegwood, Daniel Hoban, Britannia Horn, Russell House, Lauren Jankowski, Juya Jeon, Emily Kerstiens, Sarah Kilhoffer, Danielle Krug, Travis Lantz, Erich Leazer, Rachel Markowicz, Evan Martin, Barbara McAnulty, Sarah Mcginness, Matt Muskat, Eli Palm, Erin Paul, Lauren Primer, Emmy Rawson, Brittany Reyes, Carly Richards, Matthew Rodibaugh, Caleigh Roleck, Meredith Roush, Terryn Sears, Alex Smith, Gillian Smith, Reed Trende, **Francesca Whitt**, Hongji Zhang, Yi Li, Jenna Rickus, Kari Clase

Mycobacteriophages AFIS (A1) and JewelBug (A6) were isolated in Fall 2014 and Fall 2012 and selected for genome annotation in Spring 2017. Putative genes were called using the bioinformatics tools DNA Master, Glimmer, GeneMark, BLAST and Phamerator. Functions of the putative gene products were assigned based on homology to previously characterized proteins programs BLAST, HHPred and Phamerator and PECAAN was used for a quality control analysis of the final genome annotation file. Most annotated gene functions were associated with structure, replication and protection of the mycobacteriophage genetic material. Two frameshift mutations were annotated through comparative genomic analysis. One frameshift mutation was annotated in JewelBug using comparison with the tapemeasure protein for phage Jeffabunny. An additional frameshift mutation was annotated through comparison of gene 23 in AFIS and similar genes found in CactusRose, Wheeler, Bigfoot and Graduation, phages in subcluster A1. Gene products with unknown function

were also investigated using I-TASSER. Putative protein functions based on homology of protein structure by predictive structural analysis included Beta-lactamase, DNA helicase, immunity repressor, DNA and RNA polymerase, nuclease enzymes, peptidase, thioredoxin.

Seventy three phage genomes isolated from Purdue were also sequenced by Purdue Genomics Core Facility through WideSeq service. The sequenced genomes will be annotated and analyzed together with peptide data obtained using mass spectrometry. Studies have shown that GC content of mycobacteriophage genomes ranges from 50.3% to 70% and mycobacteriophages from the same cluster have similar GC content. GC content analysis will be conducted on sequenced phage genomes to examine potential correlations between the genes in the mycobacteriophage genomes and the host *Mycobacterium smegmatis* (*M. smegmatis*).

The identification of putative antibiotic resistance proteins in annotated phage AFIS suggests that mycobacteriophage could provide an alternative pathway for the spread of antibiotic resistance through a bacterial community by phage infection and replication. The GC content of AFIS, *M. smegmatis*, and *M. tuberculosis* are similar and suggests that this putative alternative pathway could provide an example of bacteriophages acting as a double-edged sword: some phages may lyse and kill the bacteria, while others may increase the virulence of bacteria. Further research into bacteriophages and their protein functions is paramount for working towards applying phage therapy in medical practice. Future collaborative work includes identification of priority candidates for determination of protein structure through wet lab experiments in the classroom.

9th annual symposium

Purdue University  
West Lafayette IN

Corresponding Faculty Member: Kari Clase (kclase@purdue.edu)



**Kari Clase**

## ***An Assessment Model for Representational Competence and Systems Thinking Embedded in SEA-PHAGES Instruction***

Kari Clase, Chandrani Mishra, Tingxuan Li, Loran Carleton Parker

HHMI SEA-PHAGES course has been widely adapted in undergraduate education and course instructors have repeatedly reported the course to have positive influence on students' learning experience. There is an opportunity to study the assessment component embedded in the instruction. In this study, we are exploring how instructional practices and assessment can inform each other to enrich the science education community, particularly, in this context, the community of HHMI-SEA-PHAGES.

One of the desired student outcomes of the SEA-PHAGES course is to think and work like a scientist. For this, students should have a clear understanding of concepts, scientific processes and should be able to communicate using representations. Representational competence enables students to understand and explain abstract scientific concepts and phenomena (Kozma & Russel, 2005). For example, in the SEA-PHAGES course, students develop a solid understanding of genome and can represent the concepts in a pencil-paper format. Therefore, we are developing an assessment model, to help instructors assess students' understanding of genome and their representational competence.

The typical instruction in the context of SEA-PHAGES involves multiple instructors, graders (i.e., teaching assistants), and classrooms (i.e., institutions). Therefore, we propose the idea of developing a coherent assessment framework that could be widely adapted by instructors of the SEA-PHAGES course. It will enable the community to achieve the following:

1) Explicitly map the content areas that students should be able to know, i.e. in assessment terminology, *claim*. In our proposed study, claims refer to students' mastery of specific learning objectives in SEA-PHAGES course.

Sample claims may include:

Students should be able to describe that...

1. *Genome is the genetic material of an organism.*
2. *Genome consists of both DNA and RNA (RNA viruses).*
3. *Genome annotation is used to identify where the genes are located and what do they code for.*

Based on claim 1, we have developed open-ended items that can be used to assess the claim.

2) Possessing a consistent grading process. This requires task-specific holistic rubrics for open-ended items developed from the previous step. Rubrics will allow the assessment results to be compared on the same metric.

Our study includes 3 phases. In the first phase, we will collect information from instructors at multiple institutions through a survey questionnaire/interview regarding learning outcomes for students in the SEA-PHAGES course. In the second phase, we will formulate our content areas of assessment framework, where these content areas will be structured as claims. In the third phase, we will generate open-ended items from the claims and develop associated item-specific rubrics. The items and rubrics in this work can be re-used by other interested institutions and to assess the same content.

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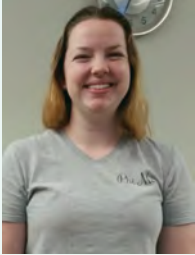


## 9th Annual SEA-PHAGES Symposium Abstract

Poster #063

Queens University of Charlotte  
Charlotte NC

Corresponding Faculty Member: Scott Weir (weirs@queens.edu)



Hailey Gase



Alyssa Strickler

### ***Queens University of Charlotte makes a “HoleInOne” discovery with “Ebony”, isolating and annotating two bacteriophages.***

Hailey Gase, Alyssa Strickler, Caroleen Ellis, Orianna Jerez, Chioma Ngene, Jennifer Easterwood, April Sipprell, Scott Weir, Joanna Katsanos

During Queens’ first year in the SEA-PHAGES program students isolated and characterized two novel phages from local soil samples. These phages were grown in the bacterial host *Mycobacterium smegmatis* mc2155 and were extracted using enriched isolation. Bacteriophage “Ebony” was identified from soil north of Charlotte and “HoleInOne” was extracted from soil on the outskirts of a golf course. Both phages were isolated, purified, and amplified using protocols from the Phage Discovery Guide as a part of the SEA-PHAGES program. The plaque size for Ebony was approximately 4mm, whereas HoleInOne plaques were less than 1mm. Transmission Electron Microscopy revealed that both phages were of the siphoviridae morphotype. After discovery and DNA isolation, Ebony and HoleInOne were sequenced at the Pittsburgh Bacteriophage Institute. Ebony is in cluster A subcluster 11 with 52,152 base pairs and a 63.8% Guanine-Cytosine (GC) content. Following auto annotation using DNAMaster 99 genes were predicted. HoleInOne is in cluster B, subcluster 2 and is 67,044 base pairs in length with a 68.9% GC content and 91 predicted genes. As a result of our inclusion in this program, student and faculty research collaborations are examining the potential use of phage therapy in *Caenorhabditis elegans*.

# 9th Annual SEA-PHAGES Symposium Abstract

Poster #64

Queensborough Community College  
Bayside NY

Corresponding Faculty Member: Urszula Golebiewska (ugolebiewska@qcc.cuny.edu)



**Makheni Jean Pierre**



**Bryan Obregon Blaker**

## ***Genomic Analysis of Mycobacterium phage Jeon***

**Makheni Jean Pierre, Bryan Obregon Blaker, Ariella Borochoy, Eric Gil Chagoya, Corey Green, Katherine Kim, David Kwun, Dwayne Lee, Stephanie Lochan, Saad Mansoor, Angie Parra Paul, Jasodra Ramdihal, Jetesh Sahadeo, Mahrukh Sohail, Lillian Talavera, Sandra Velarde, Miguel Vera, Ho Wong, Jiayi Xue, Urszula P Golebiewska**

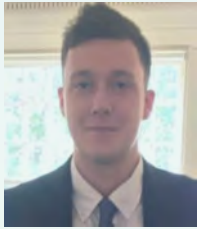
During the Spring 2017 semester, students at Queensborough Community College annotated and analyzed the genome of Mycobacteriophage Jeon. Jeon was discovered in 2013 by a student from Smith College in Northampton, MA. We were very grateful for the opportunity to annotate it. This phage is a member of the family Siphoviridae, and its morphology features a large head and a noncontractile tail. Until recently Jeon was considered a singleton, now it is placed in the new Cluster W together with Megabear and Taptic. Megabear is also from Massachusetts and Taptic is from Pennsylvania. Taptic was submitted to the Gene Bank. Jeon has a genome with a length of 60908 bps and 86 predicted genes. 85 are protein coding sequences and one tRNA for Glycine with anti-codon tcc. We used DNA master as the major tool for annotation and performed gene analyses using BLAST, HHpred, GeneMark, Starterator, Phamerator and other programs. The genome is very packed, with few long gaps and multiple instances of a few base pair overlap. The first part of the genome contains many genes with well-defined functions like helix-turn-helix DNA binding domains, terminase, portal protein, major and minor capsids, major and minor tails, tail assembly chaperones, and tail length measurement. In the middle there are LysinA, Holin, DNA recombinase, HNH endonuclease, and WhiB family transcription factor. Towards the end of the genome, there are many very short genes with unknown functions. Another feature of Jeon's genome is that all of the genes are in forward direction. BLAST of individual genes revealed that of the 85 protein coding regions in the genome, 79 are homologous with genes from Taptic, 4 with phages from Cluster B2, and one each with phages from Clusters F1 and P1. The segment similar to the Cluster B2 is exactly in the middle of the genome. The tRNA sequence is identical to that of Taptic. Megabear and Taptic both share 95% sequence identity with Jeon.

## 9th Annual SEA-PHAGES Symposium Abstract

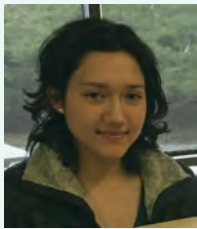
Poster #065

Rockland Community College  
Suffern NY

Corresponding Faculty Member: Jeffrey McLean (jmclean2@sunyrockland.edu)



Caleb Hughes



Caroline Peeples

### ***GingkoMaracino: Tiny Virus, Big Prospects***

Esther Jakubowicz, **Caleb Hughes**, **Caroline Peeples**, Smyrne Melissa Reveil, Valerie Schifano, Richard Twumasi-Ankrah, Ra'Vynn Waters, Jeff McLean

The Mycobacterium genus is home to human pathogens such as Mycobacterium leprae (leprosy) and Mycobacterium tuberculosis (Tuberculosis). Given the rise of antibiotic-resistance, focus has shifted to finding alternative therapies for these debilitating and often fatal diseases. Bioinformatic analysis has provided us with the tools to annotate and understand the genomes of mycobacteriophages. These tools have facilitated the search for novel genetic systems and have allowed bio-prospecting for the discovery of phages useful in treatment of these diseases. DNA master was used to annotate the genome of Mycobacteriophage GingkoMaracino. Comparative analysis software such as the PhagesDB Blast feature, Phamerator and predictive software like HHPred allowed for the identification of homologous traits with known viruses. This has revealed a plethora of interesting properties for mycobacteriophage GingkoMaracino: an endolysin system exclusive to cluster A phages, a mechanism responsible for capsid formation involving the interaction of a V-ATPase scaffolding protein with a terminase and portal protein system, phage attachment sites homologous to corresponding bacterial attachment sites of Mycobacterium tuberculosis, homology of tail proteins and GP5 region thought to be involved with phage adsorption into tuberculosis, a repressor-stoperator system which controls the switch between lytic and lysogenic behavior, and a hypothetical pathway to lysis. GingkoMaracino also encodes an exonuclease whose gene product could be utilized in creating gene knockouts, point mutations, deletions, and insertions in Mycobacterium tuberculosis. Collectively, this evidence suggests that mycobacteriophage GingkoMaracino has potential for use in the treatment and molecular manipulation of Mycobacterium tuberculosis.

# 9th Annual SEA-PHAGES Symposium Abstract

Saint Joseph's University  
Philadelphia PA

Corresponding Faculty Member: Julia Lee-Soety (jlee04@sju.edu)

**Poster #066**



**Amelia K Bielefeld**



**Sean F Collins**

## ***Exploring *Arthrobacter* bacteriophages through genome annotations***

**Amelia K Bielefeld, Sean F Collins, Melissa K Crisci, Kayla E Schulingkamp, Megan R Gentleman, Danielle M Knowles, Corinne O Merlino, Ceili S Hamill, Martyna E Habdas, Christina M Wallace, Vianca Salcedo, Iskra V Araujo, Bradley S Carpenter, Victoria L Adams, Marlyn A Henry, Leila P Benjamin, AnnaMarie E Glaser, William B O'Neill, Megan C Hartwell, Kaleigh C Williams, Julia Y Lee-Soety**

Bacteriophages that infect bacteria are a vital component in the biosphere and tools for molecular biology with possibilities in advancing medicine. Phage has lytic or temperate life cycles where the bacterial host dies soon after infection or has the phage DNA becoming incorporated into the host genome, respectively. Several important enzymes are necessary for these life cycles. A phage attaches to a host cell membrane and must also be able to break it down to injects the phage DNA into the bacterium. Then, the phage hijacks host machinery to express genes and make proteins. For the lytic cycle, phages must produce structural components, which then allow the phage particles to assemble, form progeny phages, and lyse out of the host. For the temperate cycle, several proteins are required for the incorporation and maintenance of the phage DNA into the host genome. We have been examining the complete genome sequences of four *Arthrobacter* phages from three different clusters and determining specific genes and their possible functions. TinoCrisci was isolated and characterized at SJU and belongs to the AN cluster along with three other SJU phages from last year – Mariposa, Pharsalus, and Massimo. Interestingly, TinoCrisci and Massimo produced turbid plaques suggesting that they are temperate phages while Pharsalus and Mariposa produce clear plaques being lytic phages. Phages Urla and MeganNoll were isolated and characterized at the University of Pittsburgh and belong to cluster AK. It is not known whether Urla and MeganNoll are lytic or temperate phages. We also annotated Pittsburgh phage Franzy that belonged to cluster AO' 1 and was very similar to lytic phage Brent from SJU. By comparing nucleotide and protein sequences of each potential gene to other phages in the same clusters, we have been finding that they express many common proteins at very similar regions of their genomes. Our studies have helped us gain a better understanding of phages life cycles and their diversity.



# 9th Annual SEA-PHAGES Symposium Abstract

Seton Hill University  
Greensburg PA

Corresponding Faculty Member: Kristen Butela (kbutela@setonhill.edu)

**Poster #067**



**Natalie Frydryck**



**Jordan McCready**

## ***Isolation, Characterization, and Genomic Analysis of Mycobacteriophages ActinUp (K1) and Boyle (B2)***

Alexandra Aguiar, Diana Carvajalino, John Cole, Gianna Donate, Alyssa Doyle, Jayden Emberton-Gaines, **Natalie Frydryck**, Lucas Galassi, Elizabeth Ginter, Damon Greenwald, Carrie Hardy, Kaitlyn Heslop, Sammantha Jackson, Carlie Kaltenbach, Courtney Kleist, Anitha Kunnath, Paige Lamberson, Alexa Mattivi, **Jordan McCready**, Danielle Nese, Abigail Noel, Sawyer Patrick, Elyza Pilatowski-Herzing, Yorich Poole, Mackenzie Robinson, Brittany Sharick, Mina Shenouda, Christiana Skrabak, Jenna Snyder, Abigail Squires, Holli Stiltenspole, Jannat Sumra, Nathaniel Zanoni, Kristen Butela

Mycobacteriophages ActinUp (K1) and Boyle (B2) were isolated from soil samples collected on the campus of Seton Hill University in Greensburg, PA and characterized by first-year undergraduate students participating in a one-semester combined phage discovery and bioinformatics SEA-PHAGES research course. Both phages were obtained through enrichment isolation at 25°C using the bacterial host *Mycobacterium smegmatis* mc<sup>2</sup>155, with ActinUp producing turbid plaques and Boyle producing clear plaques after 48 h incubation at 28°C, indicating potential temperate and virulent properties, respectively. The genomes of ActinUp (59.8 kb, 66.6% GC, defined linear ends) and Boyle (67.5 kb, 69.0% GC, circularly permuted) were annotated using PECAAN, DNA Master, HHPred, Phamerator, Starterator, tRNAScan-SE, Aragorn, and the Blast program suite. ActinUp contains 96 putative protein-coding genes and 1 tRNA-Trp. ActinUp is highly similar (≥95% average nucleotide identity) to previously characterized Cluster K1 phages, with the organization of the *attP* site and start associated sequences determined to be similar to other K1 phages. ActinUp gp91 and gp92 are currently under investigation for similarity to HicAB, a horizontally mobile RNA-targeting toxin-antitoxin cassette previously identified in numerous bacterial and archaeal genomes. Boyle contains 92 protein-coding genes and no tRNAs and is highly similar (≥95% average nucleotide identity) to other Cluster B2 phages. Like other Cluster B2 phages, Boyle contains a putative gene coding for lysin A (gp47) but not lysin B, and several other genes in Boyle yield BlastP matches to genes identified in *Mycobacterium abscessus* prophages.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #068

Southern Connecticut State University  
New Haven CT

Corresponding Faculty Member: Nicholas Edgington (edgingtonn1@southernct.edu)



Thomas S Hoang



Dathan D Stone

### ***Maybe A11 phages are odd: Genome annotation and comparative genomic analysis of subcluster A11 mycobacteriophages***

Thomas S Hoang, Dathan D Stone, Edward J Martinez, Bridget R Sharnick, Michael J Squires, Maurice Newton, Kara Jones, Andrea G Rizzo, Meagan M Harris,Carolynn A Keal, Edward J Martinez , Christopher P Wojtas, Zachary C Wright , Bryan Pasqualucci, Nicholas P Edgington

The “A cluster” comprises the largest cluster of discovered actinobacteriophages, consisting of almost 500 members and 18 subclusters. Since 2012, twelve subcluster A11 actinobacteriophages have been discovered, surprisingly with one-third of its members having been discovered by SCSU students. Phages have been included in a particular ‘cluster’ based on evident sequence similarity in a dot plot that spans more than 50% of the smaller of the two genomes. So this begs the question, what characteristics of the subcluster A11’s actually distinguishes them from the other A subclusters? To investigate the novelty of A11’s, we identified, studied and present data on those phams that were not only unique to A11 phages, but also were present in all A11 phages. The A11 phages also exhibit pseudolysogeny, in which their genomes are predicted to persist extrachromosomally rather than integrating into the hosts genome. ParABS systems that are present in subcluster A11 phages are responsible for this extrachromosomal maintenance, and we present a comparative genomics analysis of this system in the actinobacteriophages. Furthermore, a comparative genomic analysis of immunity repressor binding sites called ‘stoperators’ within the A cluster is presented. Finally, we present computational evidence that the A11 phages may have a novel toxin-antitoxin system.

# 9th Annual SEA-PHAGES Symposium Abstract

Southern Maine Community College  
South Portland ME

Corresponding Faculty Member: Brian Tarbox (btarbox@smccme.edu)

**Poster #069**



**Cameron C Fudge**



**Emily Haggett**



**Derek Theriault**

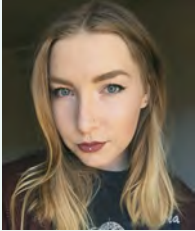
## ***Comparison of phage derived from marine and terrestrial environments***

**Cameron C Fudge, Emily Haggett, Derek Theriault**

Four strains of marine host bacteria were isolated from the North Atlantic Ocean at coordinates 43°38'47.4"N, 70°13'36.1"W. From these hosts seven separate phage were isolated. Techniques used in experimentation were similar to protocol outlined in the SEA-PHAGES laboratory manual<sup>1</sup>. Enrichment culture, streak plate isolation, PCR of the 16S rRNA gene, and simple microscopy was used to identify host genera. Four separate host genera were found, three *Pseudoaltermonas* variants and a strain of *Shewanella*. Phage were isolated from host strains. Serial dilutions were performed to isolate individual phage based on plaque morphology. Phage lysates were concentrated into medium and higher titer solutions. Phage DNA was extracted, digested, cloned, and PCR amplification of the sequences was performed. Phage samples were examined using transmission electron microscopy. Marine phage were then compared to a previously isolated terrestrial phage, Traft412, to examine morphologic and genetic differences.

Truckee Meadows Community College  
Reno NV

Corresponding Faculty Member: Tina Slowan-Pomeroy (TinaBina@me.com)



Maya D Hauk

### ***Host Specificity Testing and Multiple Sequence Alignment Analysis of Bacteriophages Guillsminger and NicoleTera***

Maya D Hauk, Christine B Emmons, Samantha Ensminger, Sarah Guill, Nicole Barnes, Tera Read, Leonard Naegele, Rebecca L Nguyen, Summer S Strickland, Tina Slowan-Pomeroy, Laura A Briggs

The goal of this research was to study host specificity on two mycobacteriophages that were isolated this year, Guillsminger and NicoleTera. Both phages were found in soil in northern Nevada. Unveiling the relationship between how bacteriophages interact with organisms and their environment allows for further insight into their ability to evolve under selective pressure. NicoleTera was found in 2015, sequenced in 2016, and annotated in Spring 2017; it is a subcluster A2 phage with 64% G/C content, containing 91 genes and six tRNAs and is 52,944 base pairs long. Guillsminger was found in 2015, sequenced in December 2016, and annotated in Spring 2017; it is a subcluster K5 with 65% G/C content, containing 94 genes, one tRNA, and is 63,153 base pairs long. Both are of the Siphoviridae morphotype. After soil collection, the bacteriophages were purified and isolated using *Mycobacterium smegmatis* mc2155 as the host bacteria. High Titer Lysates (HTLs) were prepared for each mycobacteriophage and DNA was isolated for analysis. Both phages were also imaged by transmission electron microscopy at the University of Nevada, Reno and DNA was sent to Pittsburg State University (PSU) for sequencing followed by annotation using PECAAN. Using phamerator, NicoleTera was compared to closely related A2 mycobacteriophages, Echild and ArcherNM while Guillsminger was compared to closely related K5 mycobacteriophages, Gengar and Waterfoul. Preliminary data on host specificity testing suggests that out of 27 bacterial strains Guillsminger can cross infect two, *Gordonia terrae* and *Mycobacterium smegmatis* W113. NicoleTera was shown to cross infect *Rhodococcus erythropolis* and *Mycobacterium phlei*. Multiple sequence alignment analysis is ongoing, comparing suggested host specificity genes: minor tail proteins, lysin A, lysin B, and holin.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #071

University of Alabama at Birmingham  
Birmingham AL

Corresponding Faculty Member: Denise Monti (dmonti@uab.edu)



Karly N Casey



Karina Vale

### ***Investigating Mechanisms of Infection in Newly Isolated Phage Infecting the Host, *Corynebacterium****

Karly N Casey, Karina Vale, Sameen Y Ali, Nellie N Baghaei, Sam Castillo, Aleksandra M Foksinska, Aakansha J Gosain, Nikki Mallepalli, Cameron W McPhail, Ashlyn C Murrell, Dan C Nguyen, Skye A Opsteen, Emily F Page, Madeline C Pratt, Natalie A Presado, Abby Thompson, David Saleeby, Katrina Sahawneh, Denise L Monti

Bacteriophages are the most abundant, yet least studied, entities on earth and contain a wealth of untapped genetic information for advancing biotechnology. Over 1500 full-genome phage sequences are entered in GenBank, yet only 2 corynebacteriophages had been sequenced as of August 2016. To expand the number of full-genome sequences for phages infecting the host *Corynebacterium vitaeruminis*, we isolated and characterized 12 novel corynebacteriophages from sewage influent samples. Full-genome sequencing and subsequent bioinformatics analysis of 4 phages (Darwin, PeteyPab, PotatoChip, Zion) revealed significant sequence similarity among the viruses in the EN cluster. Basic characterization experiments revealed these phages shared similar plaque and particle morphology and all were capable of calcium-independent host infection. Host-range experiments showed that 5/12 phages were capable of producing plaques on the host *C. pseudodiphtheriticum* albeit at various degrees of infectivity despite sharing sequence similarity. Here we present data describing the 12 newly-isolated novel corynebacteriophages and discuss differences in putative gene products that may be responsible for differential host infection.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #072

University of California, Los Angeles  
Los Angeles CA

Corresponding Faculty Member: Jordan Parker (jparker@microbio.ucla.edu)



Rose M Jarjoura



Stephanie Wottrich

### ***Phage Face Cream – Evaluation of Propionibacteriophage for Treatment of Acne Vulgaris and Discovery of Potential Superinfection and CRISPR Immunity Mechanism***

Rose M Jarjoura, Stephanie Wottrich, Laura J Marinelli, Jordan Moberg Parker

*Propionibacterium acnes* is a gram positive bacterium associated with acne vulgaris. Traditional antibiotic-based treatments have had limited success due to development of bacterial resistance. Phage therapy shows promise as an alternative in order to circumvent this issue. In order for *P. acnes* phage therapy to be effective, the phage must be able to infect acne-associated strains and successfully lyse target strains. In this study, two novel siphoviridae phages, Aquarius and Supernova, were isolated in order to investigate these questions. Host range assays were performed for both phages on a variety of clinical isolates of *P. acnes*. Supernova, which formed clear plaques, was able to infect acne-associated ribotype IV and V *P. acnes* strains and both Supernova and Aquarius were able to infect CRISPR-containing strains B66.8 and HLO42PA3, respectively. BLASTn revealed mutations in protospacers known to confer phage-resistance to each of these strains. In light of its broad host range and capacity to infect acne-associated strains, Supernova was mixed with Cetaphil cream, a practical vehicle to deliver phages to the skin, and streaked on *P. acnes* lawns. The phage cream, even when stored for multiple days under different conditions, showed lysis of the surrounding bacteria, indicating that topical phage treatments may be a promising therapeutic delivery method. Interestingly, the host range assay for phage Aquarius was characterized by bacterial growth in the centers of areas of clearing. Previous studies have indicated that some *P. acnes* phages may have a pseudolysogenic life cycle, which is characterized as a circularized phage genome existing as an episome within the bacterial host. Based on this, it was hypothesized that Aquarius had a potential to be a pseudolysogenic phage. Bacteria were isolated from the center of clearings and characterized as pseudolysogens capable of releasing phage. Genome circularization was confirmed by PCR and genomic sequencing. The Aquarius pseudolysogens also demonstrated superinfection immunity upon reinfection with phage Aquarius. Genome annotation of Aquarius and

Supernova revealed a putative conserved gene, gp41, which may contribute to this phenotype. A function for this protein has never been described for *P. acnes* phages, however, the protein prediction tool InterPro and the motif prediction tool MEME revealed a profoundly similar signature profile and two repeat motifs among gp41 and the lipoprotein of temperate phage (Ltp) TP-J34 (a *Streptococcus thermophilus* infecting phage). This suggests that a superinfection immunity mechanism may be present in *P. acnes* phages that would prevent efficient bacterial lysis. In conclusion, despite the success of the phage cream, the results of the CRISPR and superinfection-resistance experiments suggested that these parameters must be addressed in future endeavors for efficacious application of *P. acnes* phage-based therapeutics.

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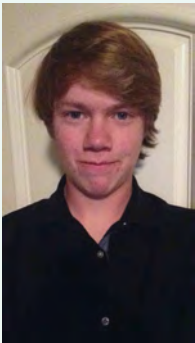
## 9th Annual SEA-PHAGES Symposium Abstract

Poster #073

University of California, San Diego  
San Diego CA  
Corresponding Faculty Member: Marcy Erb (merb@ucsd.edu)



Michelle Holland



Jason Nideffer

### ***A Survey of Antibiotic Producing Streptomyces and their Phages in San Diego Soils***

Michelle Holland, Jason Nideffer, Roland Liu, Hannah Tsunemoto, Marcella Erb, Kit Pogliano, Joe Pogliano

Our class isolated *Streptomyces platensis* strain MJ1A1 phages from 28 soil samples from around San Diego County and 40 *Streptomyces* strains from these same samples. Two phage genomes were sequenced and annotated. Phage Alvy belongs to cluster B2 and phage Dubu is only the second phage isolated belonging to cluster BJ. *Streptomyces* strains were classified by 16s rRNA sequencing and we screened 10 for antibiotic production. Most of the *Streptomyces* strains produced molecules capable of killing *E. coli*  $\Delta$ tolC and *Bacillus subtilis* PY79. We sequenced the genomes of 4 *Streptomyces* strains and used bioinformatic tools to identify potential antibiotic producing biosynthetic gene clusters. One of our strains has the biosynthetic capacity to produce over 47 unique natural products, some of which could be new antibiotics. We examined the host range of our six sequenced phages against twelve strains of *Streptomyces* and we looked for genomic differences that could explain the different host ranges.



## 9th Annual SEA-PHAGES Symposium Abstract

Poster #074

University of Colorado at Boulder  
Boulder CO

Corresponding Faculty Member: Christy Fillman (christy.fillman@Colorado.EDU)



Jack Johnson



Austin Hammermeister Suger

### ***C1 Cluster Mycobacteriophage Iota Structural and DNA Metabolism Genes Show Homology to Gordonia and Rhodococcus Phages, which Suggests a Broader Host Range for Iota***

Jack Johnson, Austin Hammermeister Suger, Erin Char, Gavin Chiem, Nathan Do, Scott Ho, Suchita Lulla, Ian McAdams, Manasa Ponnappalli

Comparative genomic analysis of mycobacteriophage Iota and Gordonia and Rhodococcus phages shows nucleotide similarity among phages infecting these diverse hosts. Using several bioinformatics tools, including Phamerator, MUSCLE alignment software, and Dot Plot analysis, we found homology in the tail tube, baseplate assembly, and DNA polymerase genes between C1 cluster mycobacteriophage Iota and two Rhodococcus phages, Finch and E3, one Gordonia phage: Pupper, and one AA cluster mycobacteriophage Phrappucinio. All five of these phages are myoviridae. During the initial steps of phage infection, the recognition of a specific bacterial host is accomplished through the docking of phage's tail fiber and base plate proteins with host cell surface receptors, and the similarity between these proteins in Iota and the Gordonia and Rhodococcus phages suggests that Iota may be able to also infect Gordonia and Rhodococcus bacteria. A broader host range in phages can impact their effectiveness in targeting specific bacteria for phage therapy and for biosensor assays.

## 9th Annual SEA-PHAGES Symposium Abstract

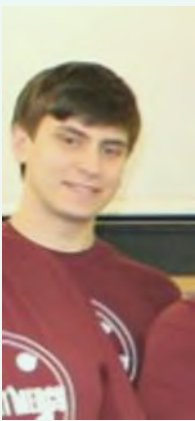
Poster #075

University of Detroit Mercy  
Detroit MI

Corresponding Faculty Member: Stephanie Conant (conantsb@udmercy.edu)



Wayman A Jones



Griffin Craig

### ***Isolation and Characterization of the Mycobacterium smegmatis specific, J-Cluster Mosaic Phage ThreeRngTarjay***

Wayman A Jones, Griffin Craig, Isabel Aklestad, Angelina Antonyan, Salar Brikho, Sarah Buhay, Nour El Yaman, Lizbeth Garcia-Leon, Zahraa Hammoud, Ali Issa, Alex Jackman, Marcel Jones, Diana McMahon, Briana Murdock, Jada Nelson, Channing Sesoko, Arren Simpson

In the Fall of 2016 the University of Detroit Mercy SEAPHAGES students isolated 8 unique Mycobacterium smegmatis specific phage. Of these, two were sent for sequence analysis and the genome data was made available for annotation by students in the Winter semester. Here we present the purification process and genome annotation data for our cluster J phage ThreeRngTarjay. ThreeRngTarjay was isolated from a soil sample found on the campus of UDM and was seen to have a unique target-like plaque morphology suggesting alternating lytic and lysogenic life cycles of this phage. It was found that the ThreeRngTarjay genome contains 113,254 base pairs and a predicted 244 genes. A total of four predicted genes (112, 129, 149, and 201) were identified as false positives based on criteria including contrasting transcription directions, excessive overlap with adjacent genes, a final RBS score highly diverging from zero, and a lack of coding potential delineated by the software Genemark and GLIMMER. One gene was added to the annotation due to coding potential found in gaps between genes 7 and 8 and containing predicted protein functional information. Of note was also a

frameshift identified in genes 30 and 31 identified as a tail chaperone protein. After final predictions were made, gene functions were assigned to those genes where function predictions could be made using BLAST data as well as HHPred software. This project is intended to help understand the genetic makeup and evolution of mosaic bacteriophages, as well as add to a growing collection of isolated and annotated bacteriophages, especially those who may have the ability to infect *Mycobacterium tuberculosis*.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #076

University of Evansville  
Evansville IN

Corresponding Faculty Member: Joyce Stamm (js383@evansville.edu)



**Humza Khan**



**Lauren Roberts**

### ***Craff-ting our Phage: Isolation and Annotation of Craff***

**Humza Khan, Lauren Roberts, Shane Bentsen, Makayla Claiborne, Gregory Maas, Anna McGriff, Émile Moura Coelho da Silva, Sanjana Sai, Brett Weinzapfel, Joyce Stamm, E. Ann Powell**

Bacteriophages, viruses that infect bacterial cells, are under active investigation because of their diverse potential applications, including as a treatment for antibiotic-resistant bacterial infections. To contribute to advancing the understanding of phage diversity and biology, and as part of our participation in the HHMI SEA-PHAGES program, we purified the phage Craff from a soil sample in a flowerbed in the Sesquicentennial Oval at the University of Evansville. Craff produces medium-sized, cloudy plaques and has a Siphoviridae morphotype. Sequencing and subsequent sequence analysis show that the Craff genome is 69,263 bp and has 66.4% GC content. Craff is a member of subcluster B1 and has 99% identity to the mycobacteriophage PG1. We auto-annotated Craff with DNA Master, using GeneMark and Glimmer predictions. Then, we compared the nucleotide sequence of all auto-annotated genes to genes in the PhagesDB and NCBI databases with BLAST. Annotations were refined with evidence from Phamerator and Starterator, and HHpred and NCBI BLAST were used to assign functions for the predicted gene products. Our analysis shows that Craff contains 102 genes, 26 of which have known functions. Craff's annotation will allow for future investigations that may include applications in agriculture, antiseptic techniques, and even medical therapies.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #077

University of Florida  
Gainesville FL

Corresponding Faculty Member: Brad Barbazuk (bbarbazuk@ufl.edu)



**Maria Ilyas**



**Thinkh Pham**

### ***Annotation of Mycobacteriophage Hurricane – a member of cluster K3***

Zacharias Anastasiadis, Rehan Bhatti, Zachary Breslow, Beau Doerr, **Maria Ilyas**, Joshua Kaiser, Annkid Milce, Stephen Monroe, **Thinkh Pham**, Guanqiao Feng, William Davis\*, Edward Braun, Brad Barbazuk

\* Washington State University, Pullman WA

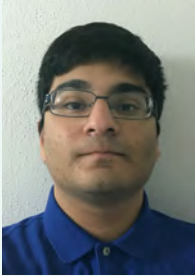
The HHMI SEA-Phages class at UF this year annotated the genome of Mycobacteriophage Hurricane, which was isolated by Karli Knudson, Miranda Edmonds and Katrina Nielsen who participated in the HHMI SEA-Phages program at Washington State University at Pullman WA. Hurricane was isolated from soil collected on the Washington State University campus. Genome sequence revealed that Hurricane is a member of the K3 cluster. Hurricane's genome is 61,318 bp in length, which is typical of K3 phages (61,252bp average). Hurricane's genome sequence is highly similar to several K3 phages, and exhibits  $\geq 97\%$  to Pixie and Tbond007, and also shows substantial similarity to members of the K6 subcluster. BlastN analysis reveals several ( $>10$ ) small gaps between the genome alignments between Hurricane and Pixie or Tbond007. Hurricane's genome has been annotated to identify 98 protein coding features, all but three of which are encoded by the forward strand. The three reverses orientation genes occur approximately mid-way through the genome and split the forward orientation genes into three groups. Details of the genome characterization of Hurricane are discussed, including an examination of potential promoters, repeats and an analysis of Hurricane genes not present in other members of the K3 subcluster.

## 9th Annual SEA-PHAGES Symposium Abstract

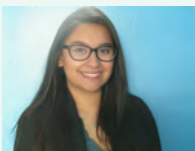
Poster #078

University of Houston-Downtown  
Houston TX

Corresponding Faculty Member: Sanghamitra Saha (sahas@uhd.edu)



**Muhammad Abdullah**



**Erica Banda**

### ***Isolation and Annotation of Cluster C and N phages***

**Muhammad Abdullah, Erica Banda, Rachna Sadana, Sanghamitra Saha**

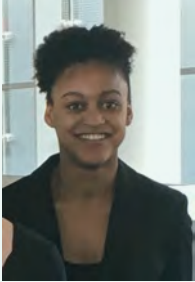
As part of the SEA PHAGES program at The University of Houston-Downtown, undergraduates took the General Biology lab I and II courses, in which mycobacteriophages were isolated and annotated. In the Biology lab I, students gathered soil samples from around the greater Houston area. Various soil samples were processed using enrichment method, and phages were isolated using serial dilution method. Isolated phages were amplified, and lysates were collected to isolate genomic DNA. Genomic DNA of the isolated phages was extracted and sequenced. Genome annotations of bacteriophages namely Purgamenstris, and Bigswole was carried out in Spring 2017 as part of Biology lab II course. Purgamenstris belonged to Cluster N, with a genome of 42,595 bp. Bigswole, belonged to Cluster C, and was found to contain the largest genome of 156514 bp. Based on plaque morphology, Bigswole appears to be a temperate phage. Genome Annotation was done employing various algorithmic software such as the Phages Evidence Collection Annotation And Networking (PECAAN), DNA Master, and Phamerator. Using these programs, putative genes were predicted and compared with homologous genes of phages. Genes with predicted functions of integrase, lysin, glycosyltransferase and minor tail proteins were identified. As these courses further progress at UHD, there is much hope that bacteriophage therapy be incorporated to combat antibiotic - resistant bacterium.

## 9th Annual SEA-PHAGES Symposium Abstract

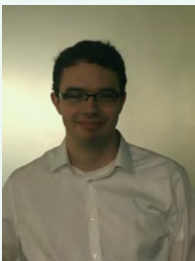
Poster #079

University of Kansas  
Lawrence KS

Corresponding Faculty Member: Rob Ward (robward@ku.edu)



Marissa Duckett



Samuel Imel

### ***Two studies on immunity: Thibault as an exception to homoimmunity in J cluster phages and an attempt to identify phage communication in M. smeg***

Marissa Duckett, Samuel Imel, Shantece Gonzalez, Eleanor Stewart-Jones, Robert Ward

During the 2015–16 academic year, the University of Kansas SEA-PHAGES class isolated and characterized the temperate J-cluster phage HokkenD. In conducting immunity tests on a HokkenD lysogen, we found that another J-cluster phage, Thibault, was able to infect the lysogen, whereas several other J-cluster phages were not. Picking up from that observation, the 2016–17 SEA-PHAGES class created an additional J-cluster lysogen with the phage Courthouse. Thibault was again able to infect this lysogen, whereas HokkenD and other J-cluster phages were not. To investigate the nature of Thibault's unusual immunity, we first compared the sequence of the repressor proteins within the J cluster. Thibault gene 81 encodes the immunity repressor and is identical to the immunity repressor in all closely related J-cluster phages including HokkenD. Immediately upstream of the repressor is a 281 bp non-coding sequence that is highly conserved in all J-cluster phages. Interestingly, there are two nucleotide substitutions in Thibault that are not found in any other closely related J-cluster phages. We suggest that this region is the operator and that these substitutions prevent other J-cluster phage immunity repressors from binding to the Thibault operator region. A challenge to this model is that Thibault itself appears to be temperate. We would like to perform site-directed mutagenesis experiments

in the future to test this model. In another project, we set out to investigate Mycobacteriophage communication. It was recently shown that temperate *Bacillus* species communicate by secreting small peptides that are imported into other bacteria and can interact with phages that have recently infected the host. We used bioinformatics to search for similar phage genes in a set of temperate Mycobacteriophages. To address the idea experimentally, we attempted to create an OppC-D deletion in *M. smeg* to test if this mutation would affect the lytic/lysogenic life cycle choice of temperate phages. Although we were unsuccessful at making the strain ourselves, we were able to get an OppD mutant strain from the University of Pittsburg and used it to test lysogeny in 8 different temperate phages. Although none of these phages (nor D29) infect the strain thus far, we are trying to work through this problem in order to conduct these tests.

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University of Louisiana at Monroe  
Monroe LA

Corresponding Faculty Member: Ann Findley (afindley@ulm.edu)



**Ann M Findley**

### ***Potential of Mycobacteriophage as Candidates for Phage Therapy - Identification of Phage that Infect Nontuberculosis Mycobacteria Pathogens***

Katelyn Setzer, Lucy Darakjian, Christopher R Gissendanner, **Ann M Findley**

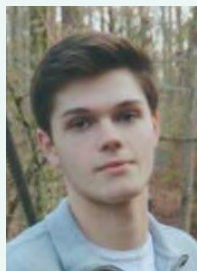
Nontuberculous mycobacterial (NTM) infections cause a wide range of debilitating diseases and are increasing in prevalence in the U.S and worldwide. With the increased resistance to antibiotics by NTM pathogens, phage therapy is gaining attention as an alternative for the treatment of such infections. Over 7,000 bacteriophages that infect the non-pathogenic species *Mycobacterium smegmatis* have been isolated. Host-range tests have identified specific subclusters of phage that can infect multiple *M. smegmatis* strains and a non-pathogenic strain of *M. tuberculosis*, indicating that they may exhibit broad host range and can possibly infect NTM pathogens. Members of the A3 subcluster have multiple phage that are potentially broad host range. In addition, these phage share a specific variant of a gene (GP5) encoding a putative minor tail protein. We have tested the ability of a large series of A3 phage to infect three pathogenic *Mycobacterium* species (*M. abscessus*, *M. chelonae*, and *M. fortuitum*) to determine if A3 broad host range extended to NTM species and if the infectivity correlates with the gene variant. The A3 phage Rockstar and Vix display significant broad host-range infectivity for one or more of these NTM hosts. In addition, as part of the SEA-PHAGES broad host-range project we have extended our study to thirty non-A3 phage isolates. The Mycobacteriophage Alma (A9), Cain (K6), Jeon (W), Larva (K5), Muddy (Singleton), PegLeg (M1), SherlockHolmes (K3), Trixie (A2), Wintermute (K4), and Zoel (K2) have successfully infected one or more of these NTM hosts as well. We are currently investigating the existence of related minor tail proteins in these isolates which may correlate with their broad host-range infectivity.

# 9th Annual SEA-PHAGES Symposium Abstract

University of Louisiana at Monroe  
Monroe LA

Corresponding Faculty Member: Ann Findley (afindley@ulm.edu)

Poster #081



Justin T Netherland



Austin D Nettles

## ***Functional Analysis of CA Cluster Rhodococcus erythropolis Phage Bonanza and a Comparative Analysis of CA, CB, and Singleton Phage Using SplitsTree, Gepard DotPlot, and Phamerator***

Justin T Netherland, Austin D Nettles, Isabel M Chauvin, Sachi Dhakal, Baxter T Flor, Savanna R Gonzalez, Jacob T Harrison, Brittany D Little, Skye M Minor, Hannah L Monk, Sabnum Pudasainy, Amy R Richard, Dustin R Rouselle, Rhae E Sevin, Lina Sihamath, Baylee L Waldrop, Peyton M Zalewski, Quoc-Nam C Duong, Austin P Dicus, Mallory B Crawford, Allison M Wiedemeier, Christopher R Gissendanner, Ann M Findley

We have successfully isolated an additional seven phage that infect the *Rhodococcus erythropolis* host. Bonanza represents another CA cluster member and is most closely related to the previously-sequenced *Rhodococcus* phage Yogi. Bonanza has 46,932 bp, 70 open reading frames, three tRNAs, and a GC content of 58.8%. As in other CA cluster phage, Bonanza shows a very high-degree of sequence homology with other members of this cluster and its ORFs represent Phams that are both exclusive to the CA *Rhodococcus* phage as well as Phams that are shared with A subcluster *Mycobacterium smegmatis* phage. We provide a functional annotation of the Bonanza genome and a comparative analysis of its relationship to other CA cluster members. Additionally, we have employed the SplitsTree, Gepard DotPlot, and Phamerator visualization tools to explore the relatedness amongst all currently-sequenced CA, CB, and Singleton phage that infect *Rhodococcus erythropolis*. These groups represent clusters that exhibit a widely varying degree of sequence homology – highly conserved CA cluster, moderately conserved CB cluster, and highly divergent Singletons. Such analyses provide insight not only into the relationship between the *Rhodococcus erythropolis* phage but can point to extended comparisons between other Actinobacter phage group isolates.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #082

University of Maine, Farmington  
Farmington ME

Corresponding Faculty Member: Tim Breton (timothy.breton@maine.edu)



Eli E Michaud



Bailey E Shevenell

### ***The discovery, isolation, and annotation of the L2 cluster mycobacteriophage, Miley16***

Eli E Michaud, Bailey E Shevenell, Liesel L Krout, Mia S Comis, Madison J Davick, Kaleb J Fletcher, Carlos Berdeja, Chelsey I Frank, Sarah E Stanley, Jacob R Montgomery, Jean A Doty, Timothy S Breton

Miley16 is a novel, cluster L2 mycobacteriophage isolated from an Abbott Park soil sample in Farmington, Maine, using the host *Mycobacterium smegmatis* mc2155. Miley16 consists of 76,653 bp, 58.9% GC content, 133 genes, and 12 tRNAs. Miley16 is highly similar to most L2 phages ( $\geq 98\%$ ), but only 90% similar to Archie. A translational frameshift was identified between two tail chaperone sequences. Miley16 also contained several genes related to lysogeny maintenance and regulation, including an integrase (Y-int), an immunity repressor, excise, and CRO (control of repressor's operator). The isolation, characterization, and genome annotation of Miley16 are discussed.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #083

University of Maine, Fort Kent  
Fort Kent ME

Corresponding Faculty Member: Kim Borges (kborges@maine.edu)



Corey Henderson



Keanna Daniels

### ***Isolation and Characterization of Refuge, a Novel Cluster A12 Mycobacteriophage from a Northern Maine River***

Corey Henderson, Keanna Daniels, Lory Guerrette, Seth Hannigan, Brittanie Hodsdon, Brianna Krystek, Jane Pettit, Mikaela Paluszek, Gabi Riccardi Shortt, Angie Rossignol, Stacy Verrell, Kim Borges

Biology Students at the University of Maine Fort Kent isolated and studied nine mycobacteriophages in 2016-2017 using *Mycobacterium smegmatis* as the host. Students used direct plating and enrichment cultures to isolate their phages from soils and river sediments in northern Maine and South Carolina. All of the phages belong to the *Siphoviridae* family based upon morphology determined by electron microscopy. Genome sequencing of three phages revealed that they belong to clusters A12, B1, and K1. Refuge, the A12 phage selected for further analysis, was isolated from submerged river sediment in Maine. Refuge's genome is 53,594 base pairs long, and contains 91 protein-coding genes and one tRNA gene. Only two other phages belong to the A12 cluster, and BLASTn comparison shows that Refuge is most closely related to phage DarthPhader, a phage isolated from soil at Gonzaga University in Washington. Refuge's ability to form plaques with *M. smegmatis* at different incubation temperatures was studied. Refuge formed larger plaques at 37 °C compared to 28 °C, and did not form plaques at 42 °C. Refuge and several other phages isolated at U. Maine Fort Kent were evaluated for host range using the spot test assay with four mycobacterial hosts: *M. abscessus*, *M. brumae*, *M. fortuitum*, and MCR15, a mycobacterial isolate similar to *M. neoaurum* that was isolated from the

same South Carolina river sample as the K1 phage. None of the phages were able to lyse *M. brumae* or MCR15. Refuge showed clearing with *M. abscessus* and *M. fortuitum*, but a titer assay showed that Refuge did not form plaques with either of these hosts, and so the bacterial killing seen in the spot test was not true infection and lysis. Further work is underway to compare the genome of Refuge to the other two A12 phages, in order to gain insights into this small subcluster.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #084

University of Maine, Honors College  
Orono ME

Corresponding Faculty Member: Sally Molloy (sally.dixon@maine.edu)



Alyssa Jones



Katherine Larochelle

### ***The Highs and Lows of Phage Diversity: introducing Cuke, Flapper and SallySpecial, a singleton mycobacteriophage and two novel Gordonia phage***

Alyssa Jones, Katherine Larochelle, Maddie Burgess, Emma Freeman, Francesca Armstrong, Caleigh Charlebois, Morgan Cavagnaro, Lily Charpentier, Pierce DiMauro, Brian Elsemore, Cody Embelton, Brandon Flowers, Connor Gilson, Sara Imam, Isaac Johnson, Rashib Kamath, Sarah McCallister, Cara McKinnon, Grace Mullen, Sarah Nichols, Will Petterson, James Seuch, Max Shoneye, Kodey Silkmitter, Grace Smith, Arielle Spalla, Marc St-Pierre, Benjamin Tero, Brooke Wilson, Melissa Maginnis, Benjamin King, Keith Hutchison, Sally Molloy

Bacteriophage, generally, and Actinobacteriophage, specifically, offer a wide array of genetic and biological diversity. And so it is with the phage isolated, sequenced and annotated this year at The University of Maine. Our phage span the spectrum of size and GC content, host range and mechanisms of lysogeny.

SallySpecial is a Gordonia phage belonging to cluster DM, a newly formed cluster containing only one other member, Emperor. The SallySpecial genome is remarkably short for a tailed phage, only 15,896 bp and encoding 23 putative genes. The genome has extremely high GC content, 70.14% and a codon usage pattern consistent with that of its isolation host, Gordonia terrae. It is a true phage minimalist in that all but three of its genes, whose functions are unknown, have predicted functions necessary for either lytic or lysogenic functions. SallySpecial can form lysogens and is predicted to have an integration-dependent repressor. The attP site was identified in the 3' end of the repressor gene and is nearly identical to a 46-bp sequence in the 3' end of a lysine tRNA gene in the genomes of G. terrae and in numerous other species of Corynebacterium, Rhodococcus and Nocardia.

Cuke was isolated using Mycobacterium smegmatis as the host, but we suspect that M. smegmatis is not its normal host. Cuke is a singleton, most closely related to the R Cluster phage. It has a genome size of 68,869 bp and 135 putative genes including 2 tRNA genes. It is a circularly permuted phage and has a GC content of

49.1%, the lowest of any phage isolated using *M. smegmatis* as a host. It's codon bias does not match that of the host. Not surprising Cuke grows poorly on *M. smegmatis*. Analysis of codon usage suggests another member of the phylum Actinobacteria, possibly *Corynebacteria* may be the normal host.

The novel *Gordonia* phage, Flapper, belongs to cluster CR and has a genome length of 67,527 bp with 96 predicted genes. Flapper forms lysogens in the host *G. terrae*, but there are no genes in the Flapper genome that encode for obvious lysogeny related functions such as an integrase or *parAB* proteins. A gene cassette immediately downstream of the lysis cassette contains genes with functions that could be related to lysogeny, including *gp57*. The *gp57* predicted protein structure aligns well with a phage or plasmid associated DNA primase. It's possible that Flapper uses a novel mechanism to maintain lysogeny.

The host range of all three phage was determined on *M. smegmatis*, *M. chelonae*, *M. marinum*, and *G. terrae*. The phage were only able to infect their original isolation host. Given the codon usage of Cuke and SallySpecial and putative *attB* site for SallySpecial in genera outside of *Gordonia*, we will be performing host range experiments in species of *Corynebacterium* and *Rhodococcus*.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #085

University of Mary  
Bismarck ND

Corresponding Faculty Member: Christine Fleischacker (clfleischacker@umary.edu)



Luke M Keller



Grace P Burns

### ***Isolation and Gene Analysis of North Dakota Phage Old Ben, Maryland Phage Lulumae, and Experiments with Alternative Bacterial Hosts***

Luke M Keller, Grace P Burns, Gabriel H Boehne, Veronica R Egging, Miranda M Furchner, Jessica K Gagen, Kathleen A Hecker, Matthew D Heinert, Marriah A Iwuchukwu, Scott M Jennings, Elizabeth B Keller, Micah C Kerney, Matthew A Kuznia, Sandra E Navarrete, Isabel J Stariha, Micah D Zimmerman, Aaron Furchner, David S Ronderos, Margaret Nordlie, Madeline A Dojs, Christine L Fleischacker

Bacteriophages are viruses that infect bacterial cells and are present in many ecosystems, including the soil. During the University of Mary's inaugural year in the SEA-PHAGE program, two novel bacteriophages were discovered. Both phages were isolated, purified, and amplified, using *Mycobacterium smegmatis* mc2 155 as the host cell. Lulumae, the first phage discovered, was isolated from Maryland soil and was obtained from direct isolation. Electron microscopy and sequencing revealed Lulumae to be a subcluster B1 bacteriophage of the siphoviridae group of phages. The genome length was 68,056 bp, and the GC content was 66.6%. The second phage, Old Ben, was isolated from soil in Bismarck, ND and was isolated from enriched cultures. Electron Microscopy revealed OldBen to be a subcluster F1 bacteriophage of the siphoviridae group of phages. The genome length was found to be 57,159 bp with a 10 bp overhang, and the GC content was 61.5%. Genome analysis demonstrated that both Lulumae and OldBen have genes similar to other B1 and F1 phages, including the LysA and LysB genes. OldBen also possesses an integrase gene, similar to other F1 phages, that is involved with the lysogenic state. Further experiments were performed to determine the host specificity of these



phages. Spot tests, spot titers, and full plate plaque assay with phage titers were performed. The alternative bacterial hosts chosen for these tests were *Mycobacterium phlei* and *Mycobacterium nonchromogenicum*. These species were chosen because they fall in the genus *mycobacterium*, were readily available, and are relatively non-pathogenic. Previous studies have shown that some F1 phages infect *M. phlei* however it was observed that OldBen, the F1 phage, was not able to infect either of these new bacterial hosts, while Lulumae, the B1 phage, could infect both new species.

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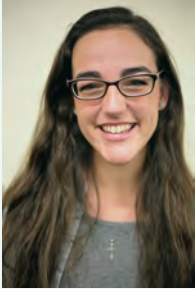


## 9th Annual SEA-PHAGES Symposium Abstract

Poster #086

University of Mary Washington  
Fredericksburg VA

Corresponding Faculty Member: Lynn Lewis (llewis@umw.edu)



Ave Keefer



Benjamin Tarnacki

### ***Phage Hunting at the University of Mary Washington***

Jacquelyn Albanese, Ryan Basham, Stacia Brooks, Lauren Closs, Meghan Darnell, Rodes Gardner, James Grigsby, Nicole Haynes, Rachel Heisner, Mary Hoffman, Jenna Johnson, **Ave Keefer**, Benjamin Moss, Brighton Payne, Marisa Payne, Gemma Spicka-Proffitt, **Benjamin Tarnacki**, Hannah Treichler, Haley Turczynski, Cat Zwemer, Jenifer Grove, Lynn Lewis

UMW Phage Hunters classes have been isolating phages from *Bacillus* hosts since 2011. Our host this year was *B. thuringiensis* subsp. Kurstaki, which has been used as microbial insecticide for pest control and is used as a simulant for *Bacillus anthracis* in biowarfare/bioterrorism studies. Of the 20 phages isolated this year, two (Cletus and Escavirius) were sequenced. Both were isolated from enriched cultures, both were myoviruses, and Cletus was found in soil from Dunn Loring, VA, while Escavirius was isolated from soil collected in Blacksburg, VA. Cletus has a genome length of 161,543 bp, which autoannotated with 298 features, a direct terminal repeat of 2,070 bp, and a GC content of 38.8%. Cletus is most similar to NotTheCreek, Kida and Hakuna by BLAST. Escavirius has a genome length of 161,795 bp, which autoannotated with 295 features, a direct terminal repeat of 2,818 bp and a GC content of 38.7% and is most similar to Megatron, DirtyBetty and SageFayge. Neither phage contained any tRNA genes when searched with tRNA Scan. During the spring semester, the class also participated in the Mycobacteriophage host range project, testing 30 phages on both *Mycobacterium smegmatis* (the original host) and *Mycobacterium aurum* (ATCC 23366). All of the phages

were able to produce plaques on *M. smegmatis*, but none of them produced plaques on *M. aurum*, indicating that these two bacteria are apparently not closely enough related for the phages isolated on one host to recognize the other. *M. aurum* does contain at least one restriction endonuclease, as identified in GenBank, but it is not known whether the restriction/modification systems in *M. smegmatis* and *M. aurum* could account for the incompatibility in hosts, or whether a receptor is missing on *M. aurum* or some other mechanism accounts for the host range restriction.

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## 9th Annual SEA-PHAGES Symposium Abstract

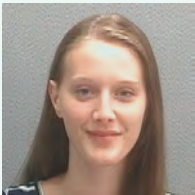
Poster #087

University of Maryland, Baltimore County  
Baltimore MD

Corresponding Faculty Member: Steve Caruso (scaruso@umbc.edu)



Bri'Anna Horne



Anna C Kawa

### ***UMBC Phage Hunters Pull a Hat Trick: Isolation and Characterization of the Tailed-Phage Triumvirate***

Amrutha Anil, Quoc Bui, Katelyn D Callaghan, Paige N Canova, Bethany Carty, Maumita Das, Claire Endres, Austin R Fowler, **Bri'Anna Horne**, Antonio J Iglesia, **Anna C Kawa**, Julia Knapo, Martin Lee, Joshua L Lockwood, Sara Mehr, Priyank Patel, Kayla M Puglisi, Eric Robins, Julianna L Sun, Joseph R Tewell, Yuliya Tkach, Shu Zhang, Matthew T Zhou, Ivan Erill, Steven M Caruso

UMBC Phage Hunters sequenced and characterized six bacteriophages as part of the 2016-17 SEA-PHAGES course. Three phi29-like *Podoviridae*: Harambe, Beachbum, and SerPounce (GenBank accessions: KY821088, KY921761, and KY947509) and a *Myoviridae*, Flapjack (KY888882) were isolated using *Bacillus thuringiensis* subsp. *kustaki* (*Btk*). Two *Streptomyces Siphoviridae* were also characterized. Abt2graduatex2 was isolated on *Streptomyces griseus* subsp. *griseus* (*S. griseus*). Scap1 is the first ever sequenced phage isolated using the potato pathogen *Streptomyces scabiei* (*S. scabiei*).

The *Podoviridae* have linear genomes ranging between 21,054 bp and 27,206 bp long ending in short, inverted repeats. BeachBum, the smallest, has 30 protein coding genes while SerPounce, the largest, has 44. No ncRNA genes have been identified. SerPounce is similar to the previously identified *Bacillus* phages Stitch, Aurora, MG-B1, and QCM11. BeachBum and Harambe are very similar phages, though quite different from previously identified phi29-like phages. The two phages did not exhibit identical host ranges, with Harambe displaying the wider range. SerPounce had one of the larger host ranges in the class, and was able to infect 80% of the *Bacillus cereus* (*B. cereus*) group hosts tested.

*Bacillus* phage Flapjack shows high sequence similarity to the C3-subcluster phages Typhen and GypsyDanger.

It has a linear chromosome 166,137 bp in length with direct terminal repeats, 288 protein coding genes, and no identified ncRNA genes. Flapjack, and other examined cluster C phages, encode an intramolecular chaperone-containing tail fiber protein showing high similarity to the one found in *Enterobacteria* phage K1F and the intramolecular chaperone domain of the *Enterobacteria* phage T5 L-shaped tail fiber as well as the PF05895 family of *Siphoviridae* minor structural proteins. Flapjack demonstrated a limited host range, infecting just half the tested *B. cereus* group hosts, fewer than 65% of other phages from the same cohort.

Abt2graduatex2 is a member of cluster BG and displays the cluster's signature prolate head. It has a 57,385 bp circularly permuted genome with 71 called protein coding genes, and shows high sequence similarity to *Streptomyces* phages Maih, TP1604, and BabyGotBac. Abt2graduatex2 carries a HicA-like toxin gene, though no corresponding anti-toxin has been identified. Abt2graduatex2 lysate was able to lyse *S. scabiei*, but was unable to produce infectious particles.

*Streptomyces* phage Scap1 is the first member of cluster B12. It has a 43,060 bp linear genome with 9 bp 3'-overhangs and 55 called genes. Scap1 lysate was able to lyse *S. azureus* and *S. griseus*, but was unable to produce infectious particles.

We report the molecular characterization and genomic analysis of these different phages, as well as comparative genomics analyses to elucidate their phylogenetic context and the function of relevant gene products.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #088

University of Nebraska-Lincoln  
Lincoln NE

Corresponding Faculty Member: Catherine Chia (cchia1@unl.edu)



Finn P Cassidy

### ***Isolation and Characterization of B1 subcluster phage Longacauda from the Univ. Nebraska-Lincoln campus***

Finn P Cassidy, Ben Ahern, Rebecca A Hartz, Jacey M Kant, Kathryn M Muilenburg, Awa Youm, Catherine P Chia

Using the host *Mycobacterium smegmatis* mc<sup>2</sup>155, the subcluster B1 phage Longacauda (Latin for long tail) was obtained by enriched isolation from soil at the base of a pine tree on the UNL campus. The circularly permuted Longacauda genome is 68,804 bp, with a GC content of 66.4%. Longacauda produces clear 1.5 mm diameter plaques with darker centers. As determined from TEM images, the average Longacauda capsid diameter was 62.5 nm and tail lengths averaged 249.4 nm. After sequencing by the Pittsburgh Bacteriophage Institute using Illumina Sequencing, students used BLASTn and DNA Master to identify a total of 105 genes that subsequently were annotated. Consistent with other B1 subcluster phages, no tRNA genes were found. With a combination of NCBI's BLASTp, HHPred, PhagesDB, Phamerator, and Starterator, the start sites of open reading frames, and their likely functions were predicted. After completing annotation tasks, students selected six Longacauda genes and their predicted functions (Lysin A, Lysin B, tape measure protein, major tail subunit, RNase E, and phospholipase A) to compare to phage within and outside of subcluster B1.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #089

University of North Georgia  
Dahlonega GA

Corresponding Faculty Member: Miriam Segura-Totten (mstotten@ung.edu)



Lauren E Colston



Grant L Zacher

### ***Isolation and genomic characterization of mycobacteriophages Donny and LilHazelnut***

Lauren E Colston, Grant L Zacher, Ryan A Shanks, Miriam Segura-Totten

Bacteriophages make up a diverse and abundant population of viruses that replicate by hijacking the cellular machinery of a bacterial host. Only a minute fraction of the phage population is characterized phenotypically or genetically. To date, this small percentage of phages represents a large diversity categorized into 88 clusters and 73 subclusters. Phage contribute to our understanding of evolution, biological diversity, and have potential medical applications. We predict that by isolating and genetically characterizing novel bacteriophages we will be able to contribute to this growing scientific field. Using *Mycobacterium smegmatis*, a non-pathogenic bacterial host, we isolated two phages, Donny and LilHazelnut, from soil samples. Donny has round, smooth edged plaques with a diameter of approximately 0.1cm. LilHazelnut has clear plaques with a slight haziness around the edges and a diameter of 0.3cm. Transmission electron microscopy determined that both phages are Siphoviridae. The DNA of each phage was extracted, and its genome sequenced. Donny, which contains 96 predicted genes, was identified as a member of the B5 subcluster of mycobacteriophages. LilHazelnut was identified as a member of the highly conserved Q cluster and was initially predicted to encode for 87 genes. However, after annotating its genome, three genes were deleted leaving 84 putative genes. Through the annotation of these two genomes, we were able to assign functions to 27 genes in Donny and 24 genes in LilHazelnut. Of the genes with assigned functionality in Donny, 96% are conserved within the B5 subcluster, while 92% are conserved outside of the cluster. On the other hand, as a testament to high conservation of the Q cluster, 100% LilHazelnut's genes with assigned functionality are conserved within the cluster. Meanwhile,

only 8.33% of these genes are conserved beyond the Q cluster. Some of the genes with assigned function include those that code for tail assembly proteins and the highly conserved hydrolytic enzymes Lysin A and B. Genetic variations in Donny and LilHazelnut are evidenced by their morphological differences. For example, the longer tail tape measure protein in Donny is partially responsible for a longer tail length for the phage. Interestingly, we found gene 70 in Donny to be truncated in relation to the corresponding genes in two of its fellow subcluster B5 phages, Acadian and Bae. Given the severe truncation of this gene, we predict that it is not essential for the phage. Subsequent research into Donny and LilHazelnut' putative protein products will help to determine the extent of variance among their domains.

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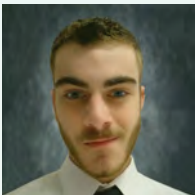
Poster #090

University of North Texas  
Denton TX

Corresponding Faculty Member: Lee Hughes (lhughes@unt.edu)



Maggie M Beard



Ian M Rapp

### ***Isolation and Annotation of 11 Streptomyces xanthochromogenes Bacteriophages***

Maggie M Beard, Ian M Rapp, Swapan Bhuiyan, Sonya R Layton, Subhayu Nayek, Lee E Hughes

Students in the SEA-PHAGES program at the University of North Texas isolated, characterized, and annotated 11 novel bacteriophages using *Streptomyces xanthochromogenes*. *S. xanthochromogenes* was isolated from soil in Japan and is not infectious to humans. Three of the phages (Oliynyk, SqueakyClean, and BeardedLady) were sequenced at the University of Pittsburgh, while the remaining phages (Amethyst, Celeste, Daudau, Diane, Esperer, Ozzie, Sebastisaurus, and Tefunt) were sequenced at UNT. Of the phages isolated, five were characterized as BD1 (BeardedLady, Celeste, Esperer, Oliynyk, and Ozzie), five as BD2 (Amethyst, Daudau, Diane, SqueakyClean, and Tefunt), and one as BB2 (Sebastisaurus). The BD1 phages had an average GC content of 66.06% and an average genome length of 50,064 base pairs. The BD2 phages had an average GC content of 66.96% and an average genome length of 50,374 base pairs. The BB phage, Sebastisaurus, was only 41,609 base pairs long and had a GC content of 62.1%. A host range was performed for each phage, testing for infection on *Streptomyces albus*, *Streptomyces azureus*, *Streptomyces griseus*, *Streptomyces venezuelae*, and *Streptomyces virginiae*. The phages were annotated and gene functions were assigned bioinformatically. One interesting function included the function of Holliday Junction Resolvase, found in three phages (BeardedLady, Celeste, and Diane). This enzyme is required to ensure proper DNA segregation after homologous recombination, facilitated by four-way Holliday Junctions (HJ's). The biological mechanisms relevant to HJ's are still being explored, but the presence of Holliday Junction Resolvases in our phage could contribute to future

research attempting to understand this process more completely. Finally, there is no conclusive evidence to suggest the presence of tRNAs in any of the phages that were isolated. One phage (SqueakyClean) appeared to contain a tRNA after a preliminary tRNA-scan. However, after further analysis using the program ARAGORN and a separate tRNA-scan, the supporting evidence was deemed insufficient, and the putative tRNA was removed from the phage genome.

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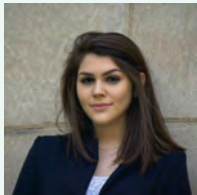
Poster #091

University of Pittsburgh  
Pittsburgh PA

Corresponding Faculty Member: Marcie Warner (mwarner1@pitt.edu)



Seohyun Im



Allison Schultz

### ***Gordonia* phages are highly diverse genetically and exhibit curious patterns in infection of different *Gordonia* species**

Seohyun Im, Allison Schultz, 2016-17 Pitt SEA-PHAGES students, Lyndsay Avery, Rebecca Bortz, Emily Furbee, Sarah Grubb, Debbie Jacobs-Sera, Wynn Meyer, Marcie Warner, Welkin H Pope, Graham F Hatfull

The majority of sequenced bacteriophage isolates infect the same host, *Mycobacterium smegmatis* mc<sup>2</sup>155. During the 2016-17 academic year, University of Pittsburgh SEA-PHAGES students isolated phages from a related host, *Gordonia terrae* 3612. Like *Mycobacterium* sp., *Gordonia terrae* is of the phylum Actinobacteria. *Gordonia* species have been implicated in foaming of sludge in wastewater treatment plants and human catheter-related infections, suggesting potential industrial and medical applications of this research. SEA-PHAGES students identified 348 *Gordonia* phages of which fifty-two were sequenced. The diversity of these phages is apparent in their variation in G+C content and genome size: G+C content ranges from 50.2% (Ronaldo and Fryberger) to 70% (Yago84) (average 62.8%) and genome size varies nearly 10-fold (smallest = Coeur at 16,223 bp; largest = SCentae at 151,316 bp; average = 66,898 bp). Diversity is also apparent when comparing nucleotide sequences of these phages' genomes; they span 12 extant clusters (CQ, CR, CS, CU, CV, CX, CZ, DB, DC, DD, DE, and DG), establish 6 new clusters (DH, DI, DK, DN, DO, and DP), and include four new singletons (Angelique, Coeur, Reyja, and Forza).

Although Reyja is classified as a singleton, a large portion of its genes are conserved and appear to be related to genes from cluster CU and CD *Gordonia* phages as well as cluster F and K mycobacteriophages. In contrast, the 192 genes of the singleton Forza are mostly orphans. The cluster that underwent the greatest expansion due to our discoveries was Cluster CS, which gained 12 additional members (20 total phages). To better characterize this rapidly expanding cluster, we tested the ability of nine CS cluster members (Anamika, BirksAndSocks, Neoevie, Woes, Hotorobo, Chelms, Gorko, Lahirium, and Boneham) to infect four additional

*Gordonia* sp. hosts: *G. lacunae*, *G. rubripertincta*, *G. westfalica*, and *G. neofelifaecis*. Six of the cluster CS *Gordonia* phages tested on alternate hosts infect all of them except *G. neofelifaecis*. However, BirksAndSocks infects all of the *Gordonia* strains tested, Lahirium infects only *G. lacunae* and *rupripertincta*, and Woes does not infect any of the alternate *Gordonia* species tested. *Gordonia terrae* phages frequently exhibit promiscuity with regard to infection of alternate *Gordonia* sp, but our results indicate that host range is variable even amongst phage grouped within the same cluster. Although minor tail proteins are frequently involved in host recognition, the only evident difference in minor tail protein pham content amongst this group of phages is the presence of pham 24769 in phages Chelms, Hotorobo, and Gorko in place of pham 20187 in the others. This difference does not correlate with the differences in infection patterns, suggesting that the unique patterns of host range observed with cluster CS phages is due to an as yet undetermined mechanism.

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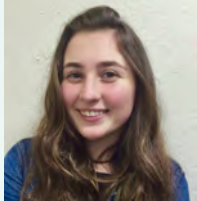


## 9th Annual SEA-PHAGES Symposium Abstract

Poster #092

University of Puerto Rico at Cayey  
Cayey PR

Corresponding Faculty Member: Edwin Vazquez (edwin.vazquez4@upr.edu)



Monica C Correa-Alfonzo



Raelys L DeLeon-Fernandez

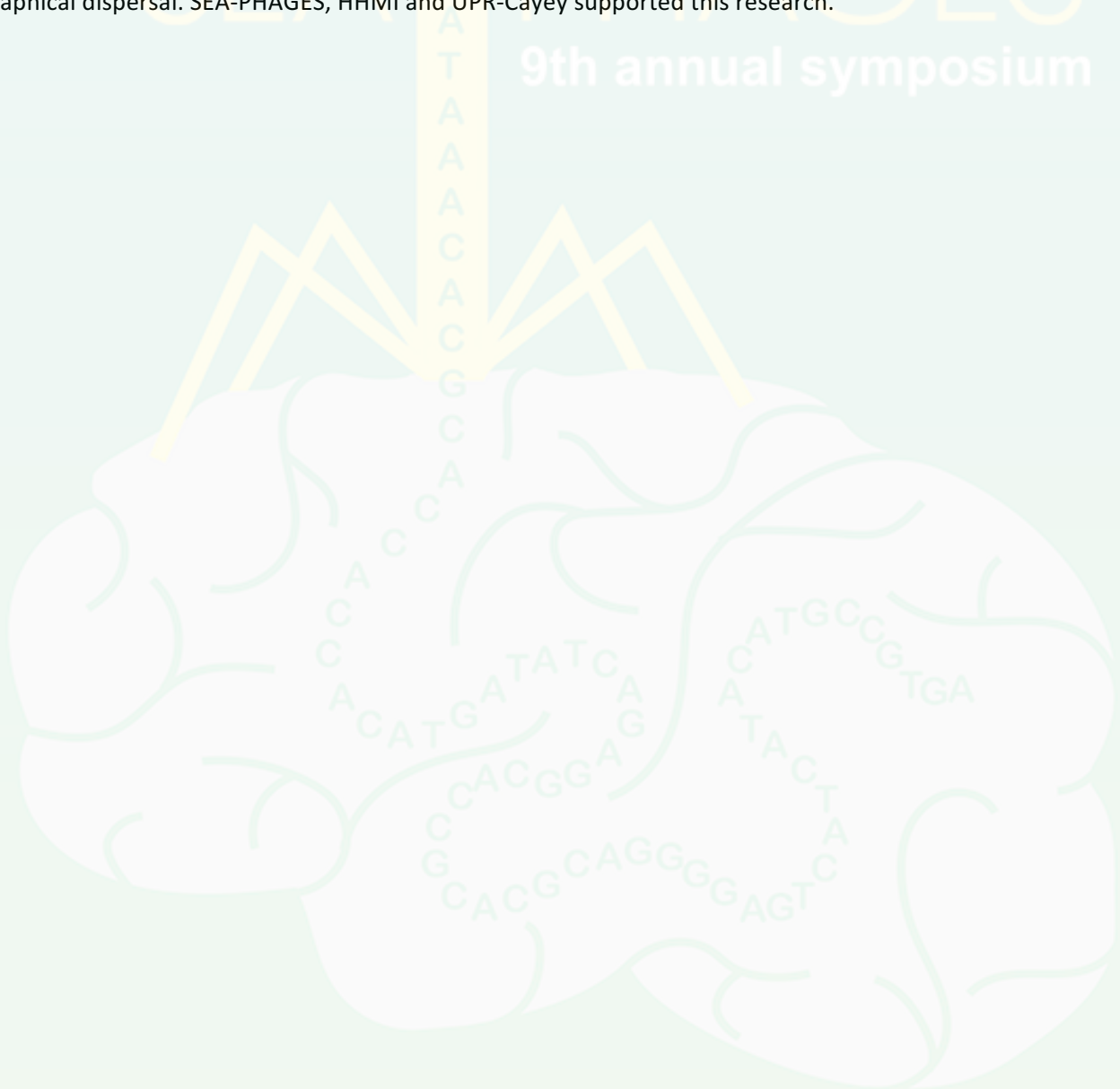
### ***Isolation and Characterization of Five *Gordonia* Bacteriophages from Tropical Environments of Puerto Rico***

Monica C Correa-Alfonzo, Raelys L DeLeon-Fernandez, Juan J Apiz-Saab, Jose C Baranda-Soto, Sofia E Berrios-Bernier, Renee Carrasquillo-Riccitelli, Nathalie Cintron-Lopez, Mariale Colon-Santos, Raul D Colon-Sierra, Jasmine Contreras-Santini, Michael E Crespo-Rivera, Lestter M Cruz-Acosta, Orlando A Cruz-Pauneto, Gabriela De Anca-Maldonado, Melody De Heusch-Lara, Ana S Delgado-Cruz, Nataly A Delgado-Hernandez, Juan E Del Valle-Pagan, Lexamalie Diaz-Garcia, Jetzalye Diaz-Perez, David A Febre-Aleman, Alexandra P Fernandez-Morales, Victoria A Figueroa-Rodriguez, Valerie D Flores-Ramirez, Gabriel A Fuentes-Cruz, Juan J Fumero-Velazquez, Jorge M Fuster-Rivera, Joel M Galindez-Couvertier, Lisa V Gonzalez-Espada, Paola D Gonzalez-Perez, Veronica I Morales-Colon, Brittany M Nieves-Ayala, Carlos E Ocasio-Caldero, Sofia I Paravisini-Domenech, Alexa D Perez-Torres, Ricardo F Pescador-Vega, Alanis P Polanco-Tapia, Alison M . Rivera-Rodriguez, Carlos E Rodriguez-Rivera, Kevin J Rosario-Rivera, Daisyree Torres-Rivera, Shadahy A Vazquez-Torres, Karla M Vega-Tirado, Mariceli Fernandez-Martinez, Michael R Rubin, Edwin Vazquez

Puerto Rico is a tropical island with rich biodiversity in its varied ecosystems, which include a rain forest, a dry forest, a karst region, mangrove forests, salt flats, coral reefs, and bioluminescent bays. This diversity is also reflected at the microbial level by the different mycobacteriophages isolated in Puerto Rico so far as part of the SEA-PHAGES program, which include clusters A, B, C, E, G, K and L. We now report on five *Gordonia* phages (using *Gordonia terrae* 3612 as a host) three of which belong to cluster CR (BiPauneto, NosilaM, and Turuncu), one to cluster DE (Tiamoceli) and one to cluster A15 (Anon). CR cluster phages have no tRNA genes, an average length of 66,950 base pairs (bp) and GC content of 65.6%. They are also rich in palindromic sequences (BiPauneto has 43 such sequences, varying from 10 to 20 nucleotides). DE cluster phages isolated so far, such as Tiamoceli, also lack tRNA genes, have an average genome size of 57,988 bp with a 67.6% GC

content. Subcluster A15, of which Anon is a member, is a crossover cluster since all the other A subclusters are made up of mycobacteriophages. These phages have an average genome size of 52,584 bp with a 62.0% GC content. Most of the genes identified so far in these *Gordonia* phages have an unknown function. We found many expected genes including some encoding structural proteins (tail and capsid) and enzymes involved in DNA replication (helicases and primases). We also identified conserved domains for a gene that codes for a peptidase transmembrane protein that acts as a bacteriocin (in CR phages) and for a gene in *Tiamoceli* encoding cutinase, which hydrolyzes cutin, a component of leaf cuticles. Bacteriophages were isolated using a simplified protocol with considerable savings in time and supplies. It involves streaking a plate with the original enriched soil filtrate, and after plaque isolation and standard purifications, preparing a second enrichment from a single purified plaque. This is then filtered again, serially diluted and from the dilution showing a web pattern (usually 100 to 10<sup>-2</sup>), a set of 10 plates is prepared to obtain an HTPL. Indigenous bacteria have been isolated from soil samples and identified as *Gordonia* by 16S rRNA PCR followed by sequence determination. Experiments are underway using these isolated *Gordonia* bacteria to explore the host range of the isolated *Gordonia* bacteriophages as well as to isolate additional bacteriophages by enrichment using these bacterial hosts. Isolation of novel bacteriophages and the diverse indigenous bacterial hosts they infect is a powerful strategy to characterize bacteriophage host range in microbial communities and its role in the evolution of genomes. The wide distribution of the bacteriophage clusters described here (some *Gordonia* CR phages were isolated from Australia), is indicative of an early evolutionary origin and subsequent geographical dispersal. SEA-PHAGES, HHMI and UPR-Cayey supported this research.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #093

University of the Sciences in Philadelphia  
Philadelphia PA

Corresponding Faculty Member: Dana Pape-Zambito (d.pape-zambito@uscience.edu)



Fariyah Miah



Victoria Pobok

### ***Phinding Genomic Gems Using DOGEMS***

Fariyah Miah, Victoria Pobok, Josie Baldwin, Nikki Bharucha, James Burkhalter, Austin Bythrow, Lauren Carter, Rakesh Chamala, Ganishah Charles-Estain, Aurora Chavarria, Mariam Dar, Abhinav Illendula, Deborah Jensen, Courtney Kovacs, Jenna Kwiecinski, Eric Levy, Ta'teanna Lyerly, Nicholas Makosiej, Nichole Mansor, Holden Ohl, Jacky Reny, Andrew Ring, Justin Robertson, Kiara Serrano, Laura Smith, Unnati Soni, Casey Theriault, Angeli Thomas, Andrew Visconti, Andre Wooley, Mariam Beshay, Norman Fultang, C. Nicole Sunnen, Dana Pape-Zambito

The University of the Sciences is an affiliate within a consortium of researchers at the Howard Hughes Medical Institute working to expand the knowledge surrounding bacteriophages. Current methods of phage isolation often select for the discovery of well-characterized phages. DOGEMS (Deconvolution of Genomes after En Masse Sequencing) is a new technique that was utilized to sequence multiple unique phages simultaneously rather than sending a single bacteriophage to be sequenced. This technique increases the likelihood of sequencing unique bacteriophages. DNA from 11 different phages was isolated and purified, then pooled together for sequencing. The result was three fully-assembled, complete DNA sequences from B2, B3 and D1 cluster phages. Using PCR and restriction enzyme digestions, the 3 unique clusters corresponded to phages Sabella, RomaT, and Visconti, all present in the original pooled sample. *In silico* studies were conducted to annotate and determine distinctive functions and proteins such as PAR B. However, based on the novelty of the clusters, many of the gene products lack known functions. These unique clusters are generally unexplored, providing an excellent opportunity for future research. Continuing to use DOGEMS in the future will allow more comprehensive study of unique bacteriophages in the future.

## 9th Annual SEA-PHAGES Symposium Abstract

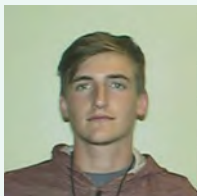
Poster #094

University of Southern Mississippi  
Hattiesburg MS

Corresponding Faculty Member: Dmitri Mavrodi (dmitri.mavrodi@usm.edu)



Savannah Underwood



Logan Ryals

### ***Discovery and characterization of bacteriophage LuckyBarnes***

Savannah Underwood, Amanda Foto, Amanda Ray, Alex Nelms, Keyshawn Kennedy, Shelby Hartley, Logan Ryals, Chandan Gurung, William D'Angelo, Dmitri Mavrodi

For several years, most colleges participating in the SEA-PHAGES program used a single bacterial host, *Mycobacterium smegmatis*. To increase the diversity of phages that infect members of the *Actinobacteriaceae*, the Phage Hunters class at The University of Southern Mississippi tested several alternative bacterial hosts. We report here the characterization of a new bacteriophage, LuckyBarnes, which was isolated from a soil sample collected in D'Iberville, MS, using enrichment with *Brevibacterium iodinum* ATCC 15728.

LuckyBarnes is a singleton siphovirus that represents a poorly characterized group of phages associated with species of *Brevibacterium*, *Corynebacterium*, and *Arthrobacter*. In the Actinobacteriophage database, LuckyBarnes is one of the only two *Brevibacterium* phages (the other is Cantare) with sequenced genomes. Electron microscopy revealed the presence of a 50-nm protein capsid and a 250-nm long, flexible tail. The phage was purified and amplified, and its DNA was isolated and sequenced using an Illumina MiSeq instrument. Single-end run reads were assembled to give a contig with 3,954-fold coverage. The 50,774-bp genome has the G+C content of 61.9%, similar to that of the host bacterium.

Putative genes in the LuckyBarnes genome were located using Glimmer and GeneMark, followed by manual inspection and annotation revision. A total of 67 protein-coding genes were identified, accounting for a 94.65 % coding capacity of the genome. Most predicted genes encoded conserved hypothetical or hypothetical proteins. Genes with assigned functions were those involved in the head and tail morphogenesis, including a major capsid protein (gp10), a terminase (gp7 and 8), a portal protein (gp9), a maturation protease (gp11), a tail-to-head connector protein (gp17), a tape measure protein (gp23), a tail subunit protein (gp19), a tail



assembly chaperone (gp21), and several minor tail proteins (gp24-27). The predicted DNA metabolism genes included a DNA polymerase (gp45), a ssDNA binding protein (gp46), a DNA primase (gp63), a DNA helicase (gp64), and three nucleases (gp47, 59, and 67). The genome encoded a lytic cassette consisting of a class II holin (gp33) and a lysin with the N-acetylmuramoyl-L-Alanine amidase activity (gp32). LuckyBarnes also carried putative genes for the dCMP-hydroxymethylase (gp41) and two glycosyltransferases (gp39, 40) that may modify its DNA and improve resistance to degradation by host nucleases. The lack of an integrase gene and clear plaques suggested that LuckyBarnes is a lytic phage.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #095

University of Texas at El Paso  
El Paso TX

Corresponding Faculty Member: Germán Rosas-Acosta (grosas3@utep.edu)



Omar Marin Sanchez



Athulya Augustine

### ***Searching for New Arthrobacter phages in the Chihuahuan Desert while contributing to the genomic annotation of other Arthrobacter phages***

Omar Marin Sanchez, Athulya Augustine, Nicole Arellano, Jessica Arredondo, Julia Cartwright, Monica Gonzalez, Dayoung Jeon, Denisse Lopez, Jacob Najera, Jacob Oropeza, Samantha Rodriguez, Sophia Villegas, Isabella Wallace, Nathiel Witter, Danielle Martinez, German Rosas-Acosta

In our previous academic year, our Phage Hunters group at UTEP attempted for the first time to isolate novel Arthrobacter phages from soil samples collected around the Chihuahuan desert, near the international border with Mexico, in West Texas and Southern New Mexico. In sharp contrast with our previous success at searching for Mycobacteriophages, for which almost every soil sample collected turned out a Mycobacteriophage, our search for Arthrobacter phages was unsuccessful and we had to expand our sampling area to the Lincoln National Forest, in Cloudcroft, New Mexico, to finally find any Arthrobacter phage. This year, the use of improved protocols for the enrichment step allowed us to successfully isolate four different new Arthrobacter phages from samples collected in our local Paso del Norte area. All newly isolated phages formed clear plaques that were easily visible after overnight incubation at 28°C. However, issues faced during the amplification of the phages, as well as during DNA purification, prevented us from being able to submit these phages for sequencing. In view of those issues, our group annotated two Arthrobacter phages previously isolated by students at the University of Pittsburgh: Edmundo, isolated from soil collected near a small stream in Pushmataha County, OK., and Fluke, isolated from soil collected next to a Baptist church in Pittsburgh, PA. Edmundo and Fluke exhibited substantial differences in their genomic organization and content, which

exemplify and reflect the apparent diversity of the Arthrobacter phages. Edmundo, a member of the AL cluster, has a 60,191 bp long genome coding for 98 different protein products, 91 of which are encoded in the forward direction, and exhibits close genomic similarity with Arthrobacter phages Laroye and Salgado (two previously characterized AL Arthrobacter phages). Fluke, a member of the AK cluster, has a 43,812 bp long genome coding for 62 different protein products, 57 of which are encoded in the forward direction, and displays substantial genomic similarity with Arthrobacter phages Immaculata, Lucy, and Preamble, all members of the AK cluster. Remarkably, in spite of exhibiting substantial differences with each other, both Edmundo and Fluke lack tRNA coding genes and exhibit high GC content (60.9% for Fluke, 64.6% for Edmundo). Further characterization of the Arthrobacter phages isolated from our local environment, currently underway, will likely allow us to gain further insights into how these phages relate to those found in areas with sharp environmental differences such as the ones annotated by our group during this academic year.

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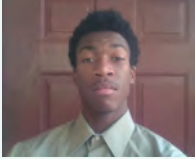


## 9th Annual SEA-PHAGES Symposium Abstract

Poster #096

University of West Alabama  
Livingston AL

Corresponding Faculty Member: Kayla Fast (kaylafast0@gmail.com)



Rakim Ali



Patricia K Schellhammer

### ***Novel J and F2 Cluster Mycobacteriophages from the Black Belt Geological Formation***

Rakim Ali, Brittany M Butcher, Joshua D Millwood, Timothy Odom, Patricia K Schellhammer, Ezekiel Ufomadu, Kayla M Fast, Tracy Keener, Michael W Sandel

Bacteriophages are viruses that infect bacteria, using them as hosts. Because these phages infect bacteria, research is being done to study their potential as alternatives to traditional antibiotics. Bacteriophages are the most numerous entity on the planet, vastly outnumbering every living organism there is, including bacteria. Due to the sheer number of bacteriophages, new phages are discovered, isolated and described often. Joining in the search for phages, the University of West Alabama has isolated and described two new phages including a new addition to the F2 subcluster called Demsculpinboyz and Schatzie a member of the J cluster. These phages were discovered using the host *Mycobacterium smegmatis* mc<sup>2</sup>155, a relative of the troublesome *M. tuberculosis*. Students followed the procedures found in the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) discovery guide to isolate and purify each phage. It is also interesting to note that these very different phages were found in such close proximity to each other. In fact, Demsculpinboyz and Schatzie were found within the same soil sample and cultured in the direct isolation step of the phage isolation process. Both of these phages proved to be housing large genomes, Demsculpinboyz has a base pair count of 57,437 and Schatzie has a base pair count of 111,345. Additionally, the content of GC nucleotides were average at 60.9% GC content in Demsculpinboyz, and 60.8% GC content in Schatzie. The number of genes in Demsculpinboyz is 117, whereas Schatzie has 201 genes; Demsculpinboyz has no tRNAs and Schatzie has 1 tRNA. Genome annotation was achieved using DNA Master, Phamerator, Starterator, HHpred, Aragorn, tRNAscan-SE, the phages database at PhagesDB, and the National Center for Biotechnology Information (NCBI) database.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #097

University of West Florida  
Pensacola FL

Corresponding Faculty Member: Hui-Min Chung (hchung@uwf.edu)



Amara Ejikemeuwa



Brett Farran

### ***King Solomon's tale – exploring ways to increase phage-hunting success and find them homes.***

Amara Ejikemeuwa, Brett Farran, Samuel Alvarado, Taylor Bowling, Molly-Catherine Brantley, Lydia Bricker, Courtney Butler, Carson Crist, Julia Dane, Sierra Hobbs, Michelle Lapak, Conner Lovell, Nicholas Ludergnani, Allison McMullen, Sohail Mirza, Hannah Perkins, Noah Thrift, Donald Vaughan, Grace Worley, May Zaw, Karen Barnes, Hui-Min Chung

Using *M. smegmatis* as the host bacteria, our phage hunting started with soil collected from sandy trail and from garden setting. While it was relatively easy to obtain phages from the garden soil, the hunting with sandy soil turned out to be really difficult. Suspecting phages in sandy soil might tend to stay in lysogenic cycles, we used several ways to induce lytic cycle by treating host cells with stress prior to the isolation step. These treatment included heat, hydrogen peroxide, pineapple juice, and UV exposure; among all, brief UV exposure seemed to be most successful. As a result, this year we isolated eight different phages: three phages from garden soil are Largelime, Kingsolomon and Nicholas, and five from sandy trail are Alectrona, Sunna, Badbeach, Tropica, and Argie. Based on the whole genome sequence information, Kingsolomon and Nicholas are L3 phages. Surprisingly these two phages share 99% identity on majority of their genomes, except Nicholas has extra 876 nucleotides in its 3' end of the genome. Kingsolomon and Nicholas also share 99% similarity on their first forty thousand or so nucleotides with other L3 phages, such as Snenia (from South Africa), Lumos (from Stuart Florida), and Clautastrophe and MsGreen (from New Orleans). To determine the cluster categories of the other six phages isolated, we took the DOGEMS approach. With limited sequence information derived from the phage DNA mixture, we were able to design cluster-specific primers for PCR gene amplification and ID the six non-genome-sequenced phages. The results indicate phage Largelime isolated

from garden soil is a L3, and phages Alectrona, Sunna and Badbeach are K3 phages, Tropica an A2 phage, and Argie a W phage. Alectrona, Sunna, Badbeach, and Argie tend to take long time to develop plaque, and their plaque size is in general small. All these 8 phages were able to infect a lysogen derived from phage Xeno of cluster N, making us thinking perhaps phages of these three clusters (A2, K3 and W) might be genetically distant from cluster N. In addition, 8 phages collected from last year, including a K1 phage Slimphazie, and Zanzibar, Marayla, Lexory, Kristannah, Xanthippeus, Phargo and Kindred of unknown clusters were also able to infect Xeno lysogen. In summary, we found the DOGEMS approach an effective way to categorize phages with limited sequence information. We highly recommend students using this approach to find their phages a home. In the future we would like to use the K3 phages (Alectrona, Sunna or Badbeach), and the W phage (Argie) to create lysogens as tools for further examining phage sensitivity and insensitivity in between different clusters.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #098

University of Wisconsin-River Falls  
River Falls WI

Corresponding Faculty Member: Karen Klyczek (karen.k.klyczek@uwrfl.edu)



John D Lowery



Caleb C Rossin

### ***A host of phages: Diversity in morphology, genomes, and host specificity exhibited by phages isolated in three Actinobacteria hosts***

John D Lowery, Caleb C Rossin, Gwenyth R Ballard, Trevor X Brammer, Jonathan M Farren, Alison M Feneis, Nicole C Firkus, Austin H Flanders, Makayla A Ford, Madison E Greenwaldt, Krista L Haglund, Ler P Htoo, Tessa A Hubbard, Collin C Heubel, Erin S Johnson, Kiana K Johnson, Danielle C Kubacki, Daniel S Lee, Selena M Neidermyer, Hayley B Revie, Megan A Richards, Ellie M Schommer, Kyle A Schroeder, Morley J Struss, Annalee R Tarras, Mariah P Troje, Madeline N Urick, Brooklynn R Williams, Amarea Y Witt, J. Alfred Bonilla, Karen K Klyczek

The UWRF phage hunting class searched for phages using three alternate Actinobacteria hosts. Each soil sample was enriched with *Arthrobacter* sp. ATCC 21022, *Gordonia terrea* 3612, and *Rhodococcus erythropolis* RIA 643. Twenty four out of 56 students (43%) observed plaques on at least one host, and several students found a phage on more than one host or had multiple phages from one host. We used cluster-specific PCR to identify phages in clusters previously isolated at UWRF, and submitted samples for sequencing that appeared to be novel. Five phages were sequenced: *Arthrobacter* phages Beans (AO) and Cheesy (AM), *Gordonia* phages SteveFrench (CS) and Brandonk123 (DE), and *Rhodococcus* phage Finch (singleton). We also submitted 16 DNA samples for DOGEMS, and received four complete sequences. By designing PCR primers specific for each sequence, we were able to identify these genomes as JayCookie (AR), Flakey (CS), Troje (CT) and Confidence (unclustered). These phages exhibit a variety of morphologies, including siphoviral and myoviral tails with lengths from 100-500 nm, and head diameters from 51-100 nm with one prolate head (Cheesy). Their genomes are also diverse, with lengths from 45,909-138,896 bp and G+C content ranging from 58.9-67.3%.

Finch has a myovirus morphology similar to *Rhodococcus* phage E3, cluster C and AA Mycobacteriophages, and cluster DO *Gordonia* phages. There is little nucleotide similarity between Finch and these genomes, but it shares 29-41 phams with each. 22 phams are shared among all of these phages, suggesting they may have a common evolutionary history.

Most of these phages appear to be lytic, but we were able to isolate potential lysogens from SteveFrench that are immune to SteveFrench and Flakey but not to the other *Gordonia* phages. No integrase gene was identified in the SteveFrench genome, but there may be one yet to be identified, or this phage uses a different mechanism for lysogeny.

As part of the host range project, we tested these phages for growth on several other Actinobacteria. Most were only able to lyse their isolation host, with a few exceptions. Cheesy appears to be able to infect *Kocuria kristinae* (NRRL B-14843) with an efficiency of plating (EOP) of  $10^{-4}$ . Also, a new phage isolated in *K. kristinae* infected *Arthrobacter* at a similar EOP. *Kocuria* and *Arthrobacter* are closely related genera and we are exploring this relationship further. Finch and other phages isolated in *R. erythropolis* infected *R. globerulus* (NRRL B-16938) at EOPs of  $10^{-3}$  to 1. We also isolated new phages in *R. globerulus* and these lysed *R. erythropolis* at similar EOPs. This collection of phages may provide new insights into host specificity.

# SEA PHAGES

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## 9th Annual SEA-PHAGES Symposium Abstract

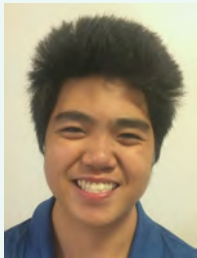
Poster #099

Virginia Commonwealth University  
Richmond VA

Corresponding Faculty Member: Allison Johnson (aajohnson@vcu.edu)



Alexa Crow



Dalton Huey

### ***Comparative genomics and functional annotation of six Bacillus phages***

Alexa Crow, Dalton Huey, Allison Johnson

As part of the 2016-2017 Phage Lab at VCU, students discovered and characterized six new bacteria-infecting viruses, or bacteriophages. Four phages were isolated using *Bacillus thuringiensis kurstaki* as the host bacteria (Janet, OTooleKemple52, Zainny, and PPisBest), and two phages were isolated using *Bacillus thuringiensis* 350 (AaronPhadgers and Bubs). These viruses are all myoviruses with a lytic lifestyle. Sequencing of the phage DNA revealed that the six genomes had lengths ranging from 159149 bp to 162692 bp with a mean GC content of 38.5%. The Spring 2017 Phage Lab focused on the computational analysis of gene sequences and functional predictions of the proteins in these phages to better understand the genetic relationships between the viruses. Students annotated the open reading frames of each genome for the best starting position and predicted function with supported database results. Comparative analysis of these six phages reveal that Janet and OTooleKemple52 can be categorized under cluster and the other four, AaronPhadgers, Bubs, Zainny, and Janet, can be grouped in another cluster. Phamerator genome comparisons, ClustalOmega protein sequence phylogeny, Splitstree protein content comparisons, and Gepard dotplot comparison support these groupings. The annotation and comparative genomics results of these six phages will be shared in further detail at the conference. For the second half of the semester, students participated in the CACAO functional annotation competition. We focused on submitting 'standard annotations' for proteins with published experimental results supporting endolysin, capsid and holin function, and then submitted 'transfer annotations' for phage protein sequences in Genbank with sufficient homology to experimentally published proteins. Combined, our work will be submitted to Genbank and Gene Ontology databases for access by other scientists.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #100

Virginia Commonwealth University  
Richmond VA

Corresponding Faculty Member: Allison Johnson (aajohnson@vcu.edu)

### ***Biochemical and structural characterization of Bacillus phage Endolysins***

Lucas Rizkalla, Louise Temple\*, Dan Nelson, Phil Mosier, Allison Johnson

\* James Madison University, Harrisonburg VA

Our story begins with a 'bioinformatics discovery' of a SEA PHAGES undergraduate student in the in silico lab course. Phage endolysins are comprised of two domains, an N-terminal catalytic domain and a C-terminal cell-wall binding domain. This student carefully characterized the sequence diversity and domain structure of a collection of Bacillus phage endolysins. We were able to identify a collaborator with endolysin expertise and move that work forward to structurally and biochemically characterize of three of these endolysins. Endolysin proteins from Bacillus phages Anthos (A54), Nigalana (N74) and TsarBomba (TB40) were expressed, purified and characterized for endolysin activity. These three proteins possess the same cell wall binding domain, but have three different N-terminal catalytic domains. Dose response curves, temperature stability, pH profiles and salt dependence were determined for all three proteins for lysis against *B. cereus* 4342. The main difference between the three enzymes is that Anthos A54 endolysin appears ~3-fold more active than the other two enzymes. Host range testing shows these three endolysin proteins behave with a similar host profile, and were able to lyse a variety of Bacillus species. Unfortunately, they do not lyse *B. anthracis*. Finally, the catalytic domains of these proteins were computationally modeled in comparison with their closest structural homologs to illustrate conservation of overall all fold as well as active site amino acids required for metal binding and peptidoglycan cleavage. This study revealed biochemical and host range similarity despite sequence and structural differences, suggesting Bacillus phages use a conserved mechanism to lyse Bacillus species.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #101

Virginia Tech  
Blacksburg VA

Corresponding Faculty Member: Stephanie Voshell (svoshell@vt.edu)



Brianna M Chop



Cameron M Myers

### ***The search for novel mycobacteriophages: Improved subcluster screening through PCR analysis of the tape measure protein gene***

**Brianna M Chop, Cameron M Myers,** Gurvina J Atwal, John B Balyozian, Ashley E Black, Zachary A Cadieux, Adam G Coffin, Kristi R DeCourcy, Jake C Deslauriers, Vikrant S Dhindsa, Sarah G Freed, Sierra E Suarez Friend, Aaron D Gardner, Kennedy Gentry, Blair J Gilmer, Casey J Gringer, Alan H Huang, Victoria N Hudson, Tyler A Jones, Mitchell C Killar, Taylor N Klein, Christian B McBride, Alexander T Nguyen, Peggy Powars, Varsha Ravichandran, Corey S Rosenblatt, Paulene A Sapao, Virginia C Saux, Inderjeet Singh, Jack R Thornton, Lisa T To, Stephanie M Voshell, Madeleine L Walters, Haoyun Yang

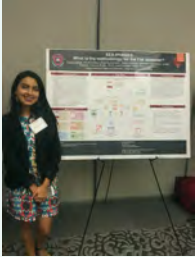
It is increasingly unlikely to find truly novel phage within the A3 and A4 subclusters since so many have been sequenced and annotated already. During the 2016-2017 academic year, students at Virginia Tech focused on finding and sequencing mycobacteriophage of less commonly studied subclusters. After isolating phage, students carried out a PCR assay of the tape measure protein gene. Data from the PCR assay was considered alongside restriction digest and tail length data to select phage that likely belonged to unusual subclusters. Mycobacteriophages Chancellor and Shaobing yielded ambiguous PCR results; genomic sequencing revealed them to be novel K4 and K1 phages, respectively. Barbarian, a suspected E cluster phage, was confirmed through sequencing. Blackmoor, a suspected A4 phage, was sent for sequencing as a control to confirm that the screening methods were accurate.

## 9th Annual SEA-PHAGES Symposium Abstract

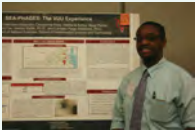
Poster #102

Virginia Union University  
Richmond VA

Corresponding Faculty Member: Carleitta Paige-Anderson (clpaige@vuu.edu)



Lizzy G Urbina



Jordan L Valentine-Alexander

### ***Isolation, Characterization and Annotation of Bacteriophages Kanely and Big3 at Virginia Union University***

Lizzy G Urbina, Jordan L Valentine-Alexander

During the 2016-2017 academic year, the Howard Hughes Medical Institute's Science Education Alliance - Phage Hunters Advancing Genomics and Evolutionary Sciences (SEA-PHAGES) course replaced one section of the traditional General Biology laboratory curriculum at Virginia Union University (VUU). Specifically, in Fall 2016, twenty students collected 40 environmental samples (both soil and water) from Central and Northern Neck, Virginia. Utilizing the bacterial host *Mycobacterium smegmatis* mc2 155, a total of 11 phages were isolated, 2 via direct isolation and 9 via enriched isolation. One representative from either isolation protocol (2 total) was selected for purification and characterization. Kanely was identified through the direct isolation method from a Champlain, VA soil sample, and Big3, another soil sample, was identified by enriched isolation from Richmond, VA. Each phage was visualized via electron microscopy exhibiting a Siphoviridae morphotype and following DNA extraction, was characterized using restriction enzyme digestion and agarose gel electrophoresis. Kanely and Big3 genomes were sequenced at the University of Pittsburg. In Spring 2017, the SEA-PHAGES class worked to annotate the genomes of both phages utilizing DNA Master genome analysis software and bioinformatics tools (including NCBI BLAST, Conserved Domain Database (CDD) and HHPred) to determine putative protein function. Both, Kanely and Big3 are members of Cluster A, and A1 sub-cluster. Kanely is 52,539 bp in length and predicted to have 93 genes, whereas Big3 is 53,442 bp in length and has 90 predicted genes. Overall, the SEA-PHAGES course-based undergraduate research experience has provided an opportunity to successfully implement an authentic research opportunity to first year students.

# 9th Annual SEA-PHAGES Symposium Abstract

Washington State University

Pullman WA

Corresponding Faculty Member: Bill Davis (wbdavis@wsu.edu)

Poster #103



Terran Gilbreath

## ***Actinobacteriophage holin protein identification and classification using protein motifs, structural analysis, and comparative bioinformatics***

Terran Gilbreath , William B Davis

Holin proteins are ubiquitous in bacteria and bacteriophage genomes. Through the process of oligomerization, these proteins create holes in the bacterial plasma membrane that release enzymes, like lysins, that catalytically break down the cell wall in preparation for phage release. A second, but independent holin function is to regulate the overall timing of cell lysis. The tertiary structure of holins is one to four alpha helices that span the bacterial plasma membrane, but due to a lack of strong conservation of primary structure, functional assignments for holin proteins remains challenging. For a large number of Actinobacterial phages in PhagesDB.org the assignment of holins is incomplete and sporadic. Saier and colleagues have used bioinformatic approaches to create seven holin superfamilies in an attempt to organize and classify most, but not all, known holin proteins. To help current and future researchers identify holins in Actinobacterial phages, we built upon the earlier work of Saier to classify the holin proteins found in SEAPhages.org. First, we identified assigned holins in phage genomes and we discovered that most holins are proximal to LysA and/or LysB genes. We next identified lysin-proximal transmembrane proteins using HHMTOP, and protein databases, NCBI Blast, and HHPred were used to identify potential holins. Holins were found in all existing Mycobacteriophage clusters and we generated a list of 37 phams that are probable holins. Thus far, the majority of holins in Actinobacterial phages are predicted to possess two or four transmembrane helices. Using the programs MEME and FIMO, we have identified protein motifs that allow for the identification of holin proteins in bacteriophage genomes. Proteins from Superfamilies IV and VII, and Family 1.E.18 have been found in phages that infect the hosts Mycobacterium and Gordonia. There are indications that proteins from Superfamilies I, II and III may be present, but we have found no evidence for holins from Superfamilies V and VI in these phages. The strongest motif in Superfamily VII proteins aligns with cytoplasmic amino acids directly upstream of a transmembrane helix, indicating that these motifs may correspond to conserved, functional regions in holins. There is no apparent conservation of the holin family in closely related bacteriophage clusters (Splitstree), indicating that holin proteins are highly variable and not strongly conserved in mycobacteriophages. In phages that contain a superfamily IV holin protein, another holin (usually from superfamily VII) is almost always found in close proximity within the genome. We hypothesize that the superfamily IV holins may have a regulatory function, but future experimental work is needed to understand if there is differential regulation of the expression and activity of multiple holin genes, or if any potential regulatory interactions exist between the expressed proteins.

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #104

Washington University in St. Louis  
St. Louis MO

Corresponding Faculty Member: Kathleen Weston-Hafer (hafer@wustl.edu)



Kelly Hartigan



Nicole Curnutt

### ***The Isolation and Annotation of Prolate-Head Bacteriophage Satis and Three Other Highly Novel Streptomyces Bacteriophage***

Kelly Hartigan, Nicole Curnutt

The Washington University in St. Louis SEA-PHAGES program isolated and annotated four novel *Streptomyces* (*lividans* and *viridochromogenes*) bacteriophages Mildred21, BillNye, Bing, and Satis. Three out of four of the studied phages have genomes larger than 125,000bp and of all *Streptomyces* phages listed in Phagesdb, Satis has the largest genome at 186,702 bp, approximately 20,000 bp longer than the next largest phage, *Mycobacteria* phage Myrna. Satis, a Singleton phage, displays many novel features and thus was chosen for in-depth study. Satis was isolated from a direct environmental sample collected from outside Danforth House on the Washington University in St. Louis campus. Satis infects bacterial species *Streptomyces lividans* producing pinpoint, cloudy plaques less than 1mm in diameter. Electron microscope data show rare, atypical physical features. Rather than the common octahedral capsid shape, Satis has a prolate head, the first of its morphology to be isolated in *Streptomyces* hosts. Its head measures, on average, 285nm by 47nm, longer than its flexible tail measuring 268nm. The genome is also quite novel in sequence, as its closest published genetic match, bacteriophage Chymera, is similar across only 15.9% of the genome (using EMBOSS Global Alignment). Satis' genome contains 324 annotated protein coding genes and 13 tRNAs. All but 33 of the proteins are orphans, leaving 291 proteins completely novel. Putative functions were called for 47 of the 324 genes, representing 14.5%. Curiously, another *Streptomyces* phage JustBecause, with almost identical prolate head morphology, was isolated in Fall 2016 concurrently with phage Satis. JustBecause has a genome length of 184,281bp and the draft DNAMaster annotation of JustBecause calls 340 protein coding genes, displaying very similar characteristics to Satis and its genome. Curiously, no tRNAs are found in JustBecause. Comparative genomic analysis between the two phages shows that 266 of their protein coding genes are considered

orthologs as judged by DNAMaster, representing about 78.5% of each's called genes. Surprisingly, comparison of the two genomes showed an ANI (average nucleotide identity) of only 74.4%, far less than predicted considering their protein, morphological, and genomic similarities. Based on the relatively high level of protein similarity yet low level of DNA identity, the two phage appear to have diverged long ago, implicating the existence of an ancient family of *Streptomyces* phage with prolate morphology. *emphasized text*

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Webster University  
Webster Groves MO

Corresponding Faculty Member: Victoria Brown-Kennerly (vbrownkennerly64@webster.edu)

## ***Kalah2: A Novel J-Cluster Actinobacteriophage***

**Kennedy Killion-Johnson**, AbdAllah Mitchell, Jacob Marsh, Shiloh Bradley, Joshua Embry, Mason Justin, Rohan Keenoy, Taylor Kissel, Richard Le, Ryan Glasgow, Kalah Collins, Mary Preuss, Victoria Brown-Kennerly

The world of viruses is still vastly undocumented and even less well understood. Through the SEA-PHAGES program, undergraduate research students discover and characterize novel bacteriophages, which are viruses that infect bacterial cells. Using the *M. smegmatis* host bacterium and a soil sample from Webster University's campus in St. Louis, MO, we isolated an apparently novel bacteriophage Kalah2 that harbors a 110,713 bp genome and is a J-cluster phage with 61% GC content, encoding 231 verified ORFs and one tRNA gene. We have used the Phamerator program as a starting point for a comparative genomics study. Of all known J phages, the JuicyJay draft genome is most closely related to Kalah2; of fully annotated phages, phage BAKA is the closest published relative via BLASTn comparison, and phage Wanda is most similar via a tBLASTx search. In our preliminary analysis of the first half of the Kalah2 genome, we have found several genomic regions that are rearranged or missing in Kalah2 in comparison to other fully annotated J-cluster phages.



Western Carolina University  
Cullowhee NC

Corresponding Faculty Member: Jamie Wallen (jamiewallen@email.wcu.edu)



Dylan Rood



Henry Salvo

## ***Preliminary Cluster Typing of 38 Mycobacteriophages Discovered at Western Carolina University and Surprisingly High Sequence Identity of a Rare Cluster M Bacteriophage***

Dylan Rood, Henry Salvo, Megan Eckardt, Jamie R Wallen, Maria D Gainey

Western Carolina University (WCU) is a member of cohort 8 and has completed two years in the SEA-PHAGES program. Over the course of two years WCU students have isolated and archived 38 mycobacteriophages. In the fall of 2016 bacteriophages Crispicious1 (A1), Galactic (F1), and IPhane7 (M1) were selected for full genome sequencing. Through bioinformatic analysis we have observed some exciting trends regarding nucleotide conservation amongst these three subclusters. Currently there are 123 A1, 119 F1, and 5 M1 genomes that have been sequenced. Blast analyses reveal that Crispicious1 and Galactic show high sequence identity (97-98%) with their top genome hits. Surprisingly, however, analysis of rare subcluster M1 bacteriophage IPhane7 has revealed that the five members of cluster M1 share higher nucleotide identity (99%) than the more frequently isolated A1 and F1 bacteriophages. In 2015 we discovered and sequenced another rarely isolate B5 mycobacteriophage (phage Serendipitous, one of only 7 that have been sequenced), and Serendipitous shares only ~90% sequence identity to other phages in the B5 subcluster. The results of the sequenced A1, F1, and B5 phages suggest that cluster M1 phages are unique in their high sequence conservation. Cluster M phages were recently described in detail by Pope et al. (2014). We will describe results of a comparative analysis of the IPhane7 genome architecture with the other four sequenced M1 genomes.

While we have been able to clearly determine the subclusters for the 6 bacteriophages that have been selected for whole genome sequencing over the past two years, we do not have whole genome sequencing data for the other 32 archived bacteriophages discovered at WCU. By combining three different methods (the Phage Enzyme Tool 2.0, tapemeasure gene PCR, and shotgun cloning) we have been able to putatively type 31 out of 32 of these bacteriophages. Currently, the most common bacteriophages discovered at WCU belong to sub-clusters A1 (37%) and F1 (24%); however, bacteriophages belonging to sub-clusters A2, A3, B1, B3, B5, E, G1, K5, and L2 have also been isolated by our students. These additional tools have allowed us to incorporate mycobacteriophage research into more advanced laboratory classes, and WCU students whose phages were not selected for sequencing get to learn to which subcluster their bacteriophage belongs. The preliminary typing of our additional 32 bacteriophages has provided a framework for understanding the diversity of phages we are discovering here at WCU.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #107

Western Kentucky University  
Bowling Green KY

Corresponding Faculty Member: Rodney King (rodney.king@wku.edu)



Megan E Dillingham



Dimond A Shelton

### ***Characteristics of Gordonia Phage Getalong (DN) and Mycobacteriophages Wachhund (F1), Squiggle (B1), Drake55 (A2), Belladonna (K1), and Morpher26 (A4).***

James K Anderson, Haley D Austin, Ethan A Brown, Margaret K Cook, Caleb W Curry, **Megan E Dillingham**, Alicia C Edds, Jaraan R Fernando, Callie A Freeman, Rukmini Ganesh, Clarissa V Gearner, Emily K Guernsey, Jacob C Harris, Olivia Hawkins, Morgan O Holt, Wren A Jenkins, Cydne C Kitchens, Chloe M Lindsey, Jack J Marquardt, Janki R Naidugari, Natalie B Ngong, Emily H Nguyen, Aidan E O'Brien, Deeya D Patel, Meghan T Perez, **Dimond A Shelton**, Tom J Shervin, Olivia C Simpson, Zachariah N Shone, Mason R Tomko, Tara N Watkins, Summer Wei, Emily M Xu, Brian Z Zhu, Amanda K Staples, Bobby L Gaffney, Rodney A King, Claire A Rinehart

One *Gordonia* phage was isolated using *Gordonia terrae* 3612 and five mycobacteriophages were isolated from *M. smegmatis* mc155. The annotations were done with the PECAAN program.

The *Gordonia* phage named **Getalong** came from an enriched compost bin in Bowling Green, Kentucky. The Getalong genome is 56,157 bp long and has 100 protein-encoding genes and no tRNAs. There are 43 genes with assigned functions. Getalong is related to three DN cluster *Gordonia* draft phages- Asapag, Horus, and Phistory.

**Wachhund** is an F1 cluster mycobacteriophage isolated from Cromwell, KY. The Wachhund genome is 54,513 base pairs long and has 98 predicted protein-encoding genes and no tRNAs. There are 47 genes with assigned functions. Wachhund is 97% identical to XFactor.

**Squiggle** is a cluster B1 mycobacteriophage isolated from Bowling Green, KY. The Squiggle genome is 68,325 bp long and has 101 protein-encoding genes and no tRNAs. There are 44 genes with assigned functions. Squiggle is 99% identical to Mana at the nucleotide level. Squiggle varies from other B1 phages in the region from 46,700 to 48,700 bp. The genes in this region of Squiggle's genome differ in pham type, length, and the number of genes compared to the corresponding region in other annotated B1 phage genomes. As typical of other B1 phages, Squiggle does not possess a frameshift within its tail assembly chaperone genes.

**Drake55** is a cluster A2 mycobacteriophage isolated from Bowling Green, KY. The Drake55 genome is 52,719 bp long and encodes 96 proteins and 1 tRNA. There are 45 genes with assigned functions. Drake55 is 97% identical to Piro94, at the nucleotide level and is a top match, for most genes, at the protein level. Drake55 gene 34 is an orpham, with no known function.

**Belladonna** is a cluster K1 mycobacteriophage isolated from Bowling Green, KY. The Belladonna genome is 59,708 bp long and encodes 94 proteins and 2 tRNAs. There are 44 genes with assigned functions. Belladonna is 99% identical to CREW at the nucleotide level. An added gene, Gene 68, is not found in other K1 phages except DrHayes, Emerson, SamuelLPlaqson, Urkel and Validus, all of which belong to pham 2971.

**Morpher26** is a cluster A4 mycobacteriophage isolated from soil samples taken in Bowling Green, KY. The Morpher26 genome is 51,294 bp in length and encodes 85 proteins and no tRNAs. There are 49 genes that have been assigned functions. Morpher26 is most closely related to the A4 phage Wile both at the nucleotide level and at the protein level. All genes also match at the pham level between Morpher26 and Wile.

9th annual symposium

## 9th Annual SEA-PHAGES Symposium Abstract

Poster #108

Winthrop University  
Rock Hill SC

Corresponding Faculty Member: Victoria Frost (frostv@winthrop.edu)



Melody C Iacino



Hallie V Smith

### ***Isolation and Characterization of Bacteriophages Asriel and HaiMas Found in the Soil at Winthrop University***

Autumn S Brewer, Shelbie A Broach, Sierra T Davis, Sara P Dixon, **Melody C Iacino**, Emily A Katsos, David N Knight, **Hallie V Smith**, Madison A Workman, Adam C Zeitz

This was Winthrop University's first year as part of the national HHMI sponsored SEA-PHAGES program. As freshman undergraduate students we isolated, purified, and characterized 11 bacteriophages found in the soil in and around Winthrop University, Rock Hill, South Carolina. Each phage was amplified in the bacterial host *Mycobacterium smegmatis* mc<sup>2</sup>155 and then characterized following DNA extraction using restriction enzyme digests and gel electrophoresis. Electron microscopy demonstrated that all eleven phages had long, flexible tails and belong to the Siphoviridae group of mycobacteriophages. After studying the electron micrographs, the digestion patterns, and quality of the DNA, two of the most potentially unusual phages, Asriel and HaiMas, were chosen to be sequenced at the University of Pittsburg. In the following semester, we continued our research by annotating both genomes using DNA Master Software and several homology search programs, including BLASTp and HHPred, to predict gene locations and determine gene function. Asriel is 74,594 bp in length, has approximately 142 predicted genes, and is a member of Cluster E. Similar to other Cluster E phages,

the Asriel genome codes for two tRNAs, and a putative immunity suppressor protein. HaiMas is 68,296 bp in length, has approximately 99 predicted genes, and is a member of Cluster B1. Both genomes encode for integrase proteins which suggest a temperate lifestyle. The SEA-PHAGES research program is helping to expand our understanding of the genomic diversity of bacteriophages found in this region of South Carolina as well as successfully introduce a genuine research experience to students in their first year as undergraduates at Winthrop University.

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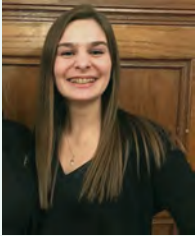


## 9th Annual SEA-PHAGES Symposium Abstract

Poster #109

Worcester Polytechnic Institute  
Worcester MA

Corresponding Faculty Member: JoAnn Whitefleet-Smith (jaws@wpi.edu)



Madeline G Manfra-Levitt



Eoin J O'Connell

### ***“We are Phamily”: Story of Two Phages with Different Homology than their Phamily***

Madeline G Manfra-Levitt, Eoin J O'Connell, Daniel B Crosby, Julia L Holtzman, Rachel L Murphy

At Worcester Polytechnic Institute, SEA-PHAGES sponsored two lab sequences: a wet lab portion to isolate bacteriophages and a bioinformatics portion to annotate the isolates' sequences. After isolating and amplifying the phages in the wet lab, four phages were sent in for sequencing for further annotation and found that they were essentially identical. Misha28 and TootsiePop were both isolated from a compost sample in Charlton, Massachusetts. After sequencing, it was found that there was a single nucleotide difference at the 11008th base pair between the two phages that was non-significant since they still code for the same proteins. Misha28 and TootsiePop were put into the F1 subcluster, but when Blasted against other phages, it was found that the second half of the genome is unrelated to the most of the other phages in the phage database. Misha28 and TootsiePop are most related to two draft phages, Awesomesauce and Piper2020, that were sequenced in 2016. Awesomesauce and Piper2020 were found in Providence, RI and Melrose, MA respectively about 50 miles from Charlton. A goal of this project was to categorize function and origin of the genes of Misha28 and TootsiePop through a comparative genomic analysis using Phamerator, ClustalW, and NCBI BLASTs. After examining the Phamerator results between Misha28, TootsiePop and Awesomesauce, it can be seen that the homology between the four sequences are nearly identical, except for a few sections in the latter half of the

sequences, after around 30,000 base pairs. This is interesting since they are all phages found in New England that happen to have similar homology that is different from the rest of the F1 phages. Additionally, the homology of the tape measure gene, one that is constant in all of the phages, was analyzed using ClustalW multisequence alignments and phylogenetic trees. It was found that Misha28's origin is different from other F1 phages and in general the other phages that seemed to have similar Blast hits to Misha28 and TootsiePop. Using these constant Blast hits via DNA Master, Phamerator was run, and it was seen that certain sections of other phages matched Misha28 and TootsiePop, but none of them were similar all the way through the genome. Tortellini is a P4 phage that matched Misha28 and TootsiePop between genes 62-63, and 65-69, whereas, BuzzLyseYear and Squirty, two F3 phages, matched Misha28 and TootsiePop between genes 45-50. SkinnyPete was an N phage that highly matched Misha28 and TootsiePop between genes 71-72 and 75-76.

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## 9th Annual SEA-PHAGES Symposium Abstract

Poster #110

Xavier University of Louisiana  
New Orleans LA  
Corresponding Faculty Member: Joe Ross (jross@xula.edu)



Lauren A Thornton



James M Vu

### ***Phaging in the Big Easy***

Claude F Albritton, Sarah C Bertrand, Shanzay S Chaudhry, Vzair A Cheema, Camilla Do, Huyen M Duong, Dalia H El-Desoky, Kelsey M Green, Rhea N Lee, **Lauren A Thornton**, **James M Vu**, Mah Noor Zahra, Joseph F Ross

Phages were isolated from a variety of locales in and around the Greater New Orleans, Louisiana area using standard microbiological techniques. When allowed to form plaques in a soft-agar overlay culture with *M. smegmatis* mc2155 as host, phages displayed a variety of plaque sizes and morphologies. Titers of lysates (plaque-forming units per milliliter) varied over several orders of magnitude. One phage, Clautastrophe, was selected for sequencing. The genome is somewhat more than 72,000bp in length, with cohesive ends showing a ten base pair overlap. BLASTn analysis reveals considerable nucleotide homology with the genomes of other known mycobacteriophages, including Snenia, Lumos Lolly9 and Whirlwind. These homologies support assignment to the L3 subcluster. DNA Master autoannotation employing Glimmer and GeneMark calls about 132 total features. Analysis with Aragorn via the World Wide Web, external to the DNAMaster environment, calls 10 tRNA-encoding genes. With one exception, these are located in a cluster towards the 3-prime end of the genome. All but one of these calls is supported as well by analysis with tRNA ScanSE. All code for standard amino acids, with no tmRNAs called. With the help of BLASTp analysis and similar tools, it is possible to make at least tentative proposals for function in the case of about twenty-five percent of protein-encoding gene calls.

# 9th Annual SEAL PHAGES Symposium Abstract

Poster #111

Brown University  
Providence, RI  
Corresponding Faculty Member: Richard Bungiro



Zachary Jordan



Fariha Kohistani



## ***Mycobacteriophage Hannaconda characterization and protocol exploration***

Zachary Jordan, Fariha Kohistani, Alex Song, Clancy Brooks, Maxwell Koslov, Quinn Matos, John Nicklas, Sarah Taylor, Richard Bungiro, Yang Zhou, Kelly Cleveland. The presenters are Zachary Jordan and Fariha Kohistani, the faculty members Sarah Taylor, Richard Bungiro, and Yang Zhou.

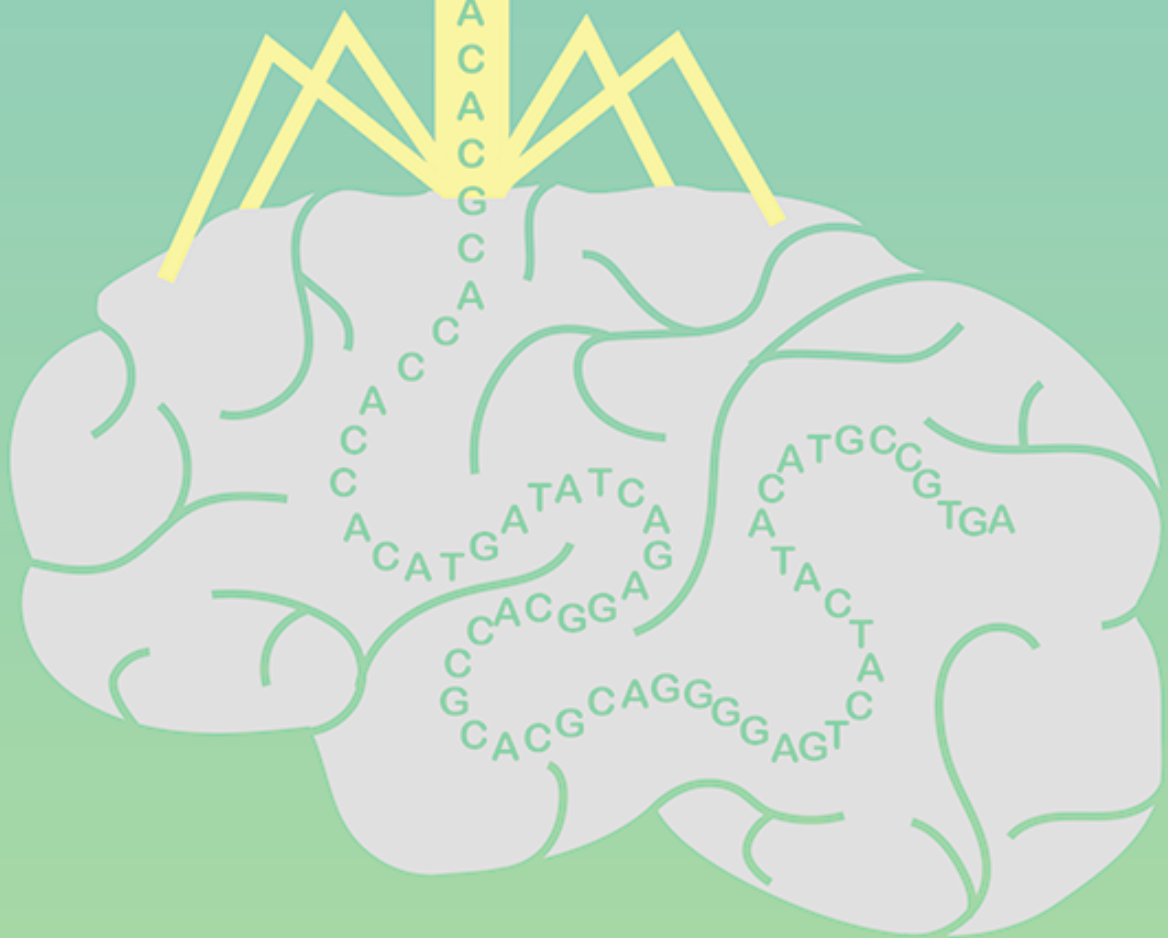
24 bacteriophages infecting *Mycobacterium smegmatis* were isolated from local environments. Hannaconda, isolated using direct plating, was chosen for genome annotation and analysis using Phamerator, Starterator, and DNA Master Genome Annotation Software. Sequencing and further study of this cluster J phage revealed that Hannaconda is 234 genes in length and contains 2 tRNA genes. Initial Phamerator data points to the existence of orphans, genes in a novel bacteriophage pham, at two positions in the genome. In addition, students conducted varied experimental wet lab work to investigate superinfection immunity in confirmed lysogens, direct phage competition dynamics, and the effects of host cell density on lysogeny.

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GENERAL INFORMATION

# 9<sup>th</sup> SEA PHAGES Symposium

June 9 – 11, 2017

## GENERAL INFORMATION

### PROGRAM BOOK & AGENDA ACCESS

This year, we're excited to announce that the program book will be completely online. You can access the Program Book and Agenda using most web-capable devices, and instructions to do so are provided below. A downloadable/printable agenda (PDF) is also available at the program website at <https://seaphages.org/meetings/22/>. Hard copies of the program book and agenda will **NOT** be available at the symposium.

1. To access the online program book and agenda, click (or copy and paste this link into your web browser): <https://crowd.cc/seasymp2017>
2. Use the QR code below



### HASHTAG

#SEAsymp2017

### ATTIRE

Attire for the entire SEA Symposium is business casual.

### PRESENTERS | TALKS

Those selected to give talks must upload their presentations to the "SEA Symposium 2017 Talks" folder in Dropbox by Thursday June 8th, 2017. Presenters will receive a link to this Dropbox folder. Please name your files as indicated in the document "Symp\_Filename", which is included in the Dropbox folder. You can continue to update your talk until 2 hours prior to your talk, working off of the file you uploaded to Dropbox.

### PRESENTERS | POSTERS

Every school is required to present one student poster at the symposium. The maximum height and width for each poster cannot exceed 48"x 48". There are two poster sessions, one for odd-numbered posters and another for even-numbered posters. Poster assignments can be found in the program book.

### POSTER JUDGING

There will **NOT** be judging of posters at this year's Symposium. All guests are encouraged to visit all of the posters, to discuss the data and to also provide poignant feedback to the presenters as guidance towards future presentations.

### LOCATION

HHMI Janelia Research Campus  
19700 Helix Drive  
Ashburn, VA 20147  
<http://www.hhmi.org/janelia>

### MEETING ROOMS & SEATING ASSIGNMENTS

All talks will be presented in the Auditorium. Talks will also be projected in the Seminar Room or Synapse Meeting Room, which are also equipped with microphones and video capabilities.

- All students are assigned to the Auditorium for talks throughout the symposium.
- All faculty all assigned to the Seminar Room for talks throughout the symposium. A rotating subset of faculty will be assigned to the Auditorium or Synapse Meeting Room. Faculty should review their seating assignments on the name badges before each session.

### MEALS

All meals will be provided at Janelia Research Campus. Those observing Ramadan should inform SEA Staff during check-in, and boxed meals will be provided.

### LODGING INFORMATION

Lodging assignments were provided to participants when travel plans were made. Participants will be lodged at one of the hotels below:

- Janelia Research Campus (Janelia) –19700 Helix Dr, Ashburn, VA, 20147 – 571-209-4000
- DoubleTree by Hilton Hotel Sterling-Dulles Airport (Doubletree) – 21611 Atlantic Boulevard, Sterling, Virginia, 20166 – 703-230-0077
- Holiday Inn Dulles International (Holiday Inn) - 45425 Holiday Drive, Dulles, Virginia, 20166 – 703-230-0077

### SHUTTLE BUS SCHEDULE

Shuttle buses will be provided for participants. All participants are required to use the shuttle buses during their published operating hours. The shuttle bus schedule can be found in the online program book, online at <https://seaphages.org/meetings/22/>, in the attached downloadable/printable agenda (PDF), and at the end of this message.

### PARKING

Parking at Janelia Research Campus is available **ONLY** for overnight guests staying at Janelia, and for day guests. Overnight guests staying at the DoubleTree and Holiday Inn hotels must leave their cars at the hotels and use the shuttle busses provided from the hotels to Janelia.

### REIMBURSEMENT OF EXPENSES

Participants are expected to cover the cost of incidentals (e.g. meals during travel, baggage fees, or lodging not required for the meeting). HHMI will NOT reimburse you for travel insurance, personal charges such as entertainment, or taxis to tour D.C. or visit friends.

If you believe special circumstances justify reimbursement, please contact Billy Biederman at [sea@hhmi.org](mailto:sea@hhmi.org). In such cases, it is important that you keep all receipts and travel stubs.

# 9<sup>th</sup> SEA-PHAGES Symposium

## SHUTTLE INFORMATION

Reston Limousine shuttle buses will operate from: Dulles to Janelia; Dulles to DoubleTree; Dulles to Holiday Inn.

Upon arrival at Dulles Airport, proceed to Baggage Claim Area, Carousel #3.

Look for a Reston Limousine representative holding a sign for HHMI.

The representative will direct you to either the bus going to Janelia, Holiday Inn, or the DoubleTree

### ARRIVALS – Friday, June 9

#### SHUTTLES – Dulles to Janelia, Dulles to DoubleTree, and Dulles to Holiday Inn

Depart at:

12:00 PM

1:15 PM

2:30 PM

3:45 PM

#### Additional Shuttle – Dulles to Janelia

Depart at 5:00 PM

#### SHUTTLES – DoubleTree to Janelia, and Holiday Inn to Janelia

Depart at:

2:30 PM

3:30 PM

4:30 PM

Guests staying at the DoubleTree or the Holiday Inn can also use the hotel shuttle from Dulles to their assigned hotel.

### EVENINGS – Friday and Saturday

#### SHUTTLES – Janelia to DoubleTree, and Janelia to

#### Holiday Inn

Depart at:

9:00 PM

9:30 PM

10:00 PM

10:30 PM

11:00 PM

### MORNINGS – Saturday and Sunday

#### SHUTTLES – DoubleTree to Janelia, and Holiday Inn to

#### Janelia

Depart at:

6:45 AM

7:00 AM

7:15 AM

7:30 AM

7:45 AM

8:00 AM

### DEPARTURES – Sunday, June 11

#### SHUTTLE – Janelia to Dulles, and Janelia to Reagan National Airport (DCA)

Depart at 1:00 PM

#### SHUTTLE – From Janelia to HHMI HQ (for Genome Announcement Workshop Guests Only).

Depart at 2:00 PM

Additional transportation information will be posted on the departure list at the registration desk.

# 9<sup>th</sup> SEA-PHAGES Symposium

## SYMPOSIUM & HOTEL LOCATIONS

### SYMPOSIUM

HHMI Janelia Research Campus  
19700 Helix Drive  
Ashburn, VA 20147  
<http://www.hhmi.org/janelia>

### HOTEL LOCATIONS

**Janelia Research Campus (Janelia)**  
19700 Helix Dr,  
Ashburn, VA, 20147  
*Tel. 571-209-4000*

**DoubleTree by Hilton Hotel Sterling-Dulles Airport (Doubletree)**  
21611 Atlantic Boulevard,  
Sterling, Virginia, 20166  
*Tel. 703-230-0077*

**Holiday Inn Dulles International (Holiday Inn)**  
45425 Holiday Drive,  
Dulles, Virginia, 20166  
*Tel. 703-230-0077*