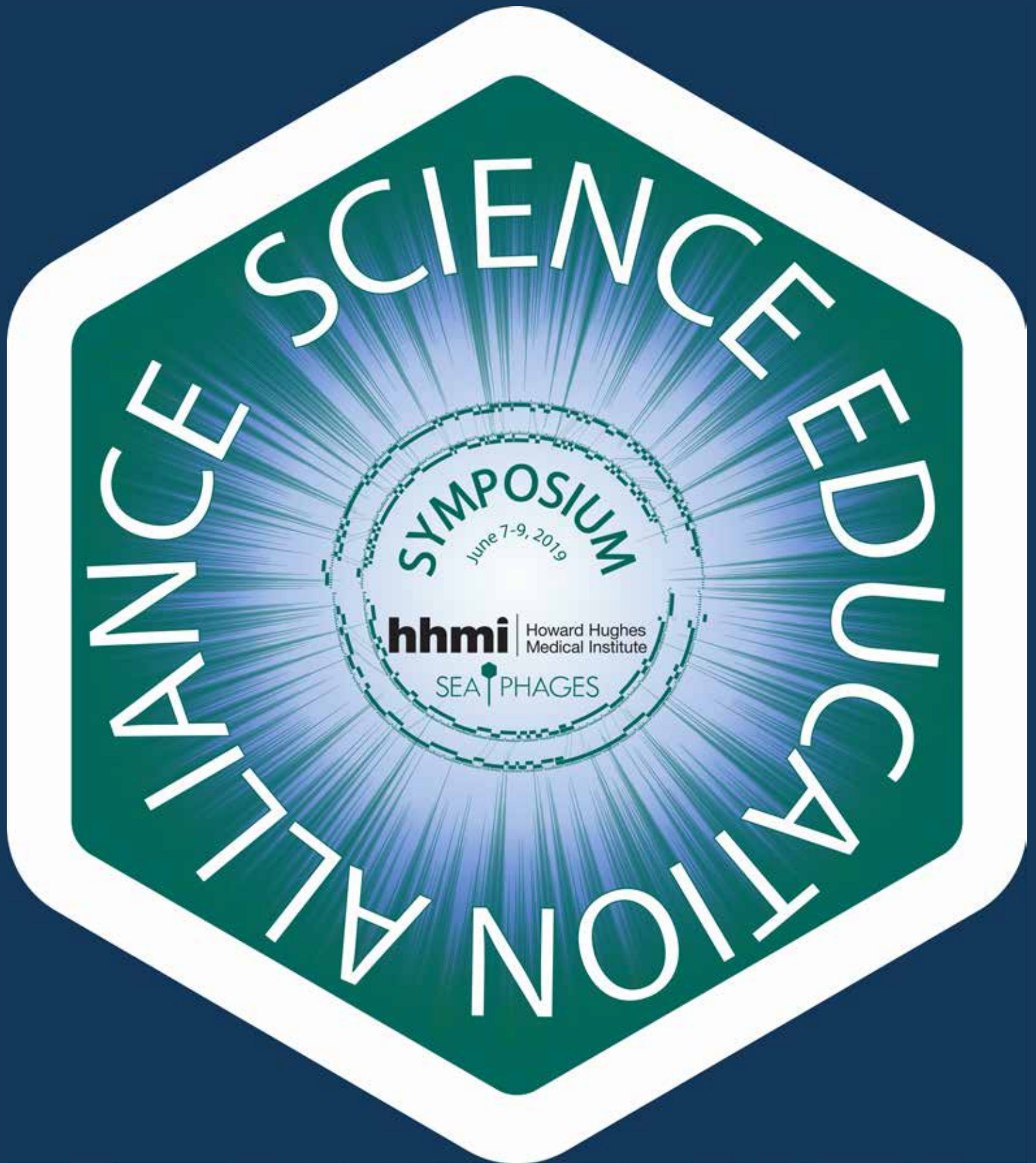


# PROGRAM BOOK



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# 11<sup>TH</sup> SEA-PHAGES SYMPOSIUM

Friday, June 7, 2019

3:00 PM – 4:30 PM	CHECK-IN POSTER SET-UP STUDENT PRESENTER PRACTICE PERIOD	RECEPTION DESK/GALLERY LOBBY AUDITORIUM
4:45 PM – 6:15 PM	STUDENT DEBRIEFING	AUDITORIUM
5:00 PM – 6:00 PM	FACULTY SOCIAL & ANNOUNCEMENTS	LOBBY
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. Pardis Sabeti Harvard University & HHMI	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
8:45 PM – 9:30 PM	Social	LOBBY
9:00 PM – 11:00 PM	SHUTTLES TO NATIONAL CONFERENCE CENTER (NCC) – EVERY 30 MINS	DRIVEWAY LOOP ENTRANCE

*The shuttle service is for guests that are staying at the NCC.*

# 11<sup>TH</sup> SEA-PHAGES SYMPOSIUM

Saturday, June 8, 2019

7:00 AM – 8:15 AM	BREAKFAST	DINING ROOM
<i>NCC guests will be shuttled from the NCC to Janelia Research Campus.</i>		
8:30 AM – 8:45 AM	INTRODUCTORY REMARKS	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
8:45 AM – 10:15 AM	ORAL PRESENTATIONS I MODERATOR: SIMON WHITE	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM

**ANNOTATION OF PHAGE MCGALLEON FROM SUBCLUSTER EA1 IN MICROBACTERIUM FOLIORUM**

ELIZABETH HAMPTON & DANIELLE DAVIS - COLLIN COLLEGE

**NOVEL BACTERIOPHAGES AGAINST ENVIRONMENTAL PHACTORS – PHAGE STABILITY PUT TO THE TEST!**

NICHOLAS BARBIERI & ERIN GALLAGHER - DREXEL UNIVERSITY

**ISOLATION AND CHARACTERIZATION OF MICROBACTERIUM PHAGE ETTA AND DISCOVERY OF BACTERIOPHAGE VERS USING A NOVEL ANTARCTIC CRYOBACTERIUM ISOLATE**

KELSEY LEACH - MINNESOTA STATE UNIVERSITY MOORHEAD

**ISOLATION OF MYCOBACTERIOPHAGES FROM SEWAGE**

EMILEE L CARR & ROCHELLE GAERTNER - BRIGHAM YOUNG UNIVERSITY

**A TALE OF DOGEMS – LESSONS LEARNED OUT OF M. FOLIORUM PHAGE HUNTING**

CASSANDRA KELSO & NAOMI SEMAAN - UNIVERSITY OF WEST FLORIDA

**MYCOBACTERIA ACHIENSE PHAGES: CONTINUING CHALLENGES AND UNSOLVED PUZZLES**

TAIANA JAMES & SOLENE SOSSAH - COLLEGE OF WILLIAM & MARY

10:15 AM – 10:30 AM	BREAK	LOBBY
10:30 AM – 12:30 PM	POSTER SESSION I	LOBBY
12:30 PM – 1:30 PM	LUNCH	DINING ROOM

# 11<sup>TH</sup> SEA-PHAGES SYMPOSIUM

Saturday, June 8, 2019

1:30 PM – 3:00 PM      ORAL PRESENTATIONS II      STUDENTS IN AUDITORIUM  
MODERATOR: ELVIRA EIVAZOVA      FACULTY IN SEMINAR ROOM

**IDENTIFICATION OF TWO GORDONIA PHAGES: STEAMEDHAMS AND SUERTE**

JOHN PERKINS & LAUREN PINCUS - GEORGE MASON UNIVERSITY

**A NOVEL APPROACH TO IMPROVING AUTOMATED BACTERIOPHAGE GENOME ANNOTATION UTILIZING MACHINE LEARNING**

ELISE RASMUSSEN - JAMES MADISON UNIVERSITY

**EXPLORING A SEA OF PHAGES: NEW INSIGHTS INTO PHAGE GENOME DIVERSITY, HOST-PHAGE INTERACTIONS, AND IMMUNITY RELATIONSHIPS**

CAITLIN MURPHY, GRACE CIABATTONI - LEHIGH UNIVERSITY

**IMMUNITY TESTING AS A PROBE FOR PHAGE DIVERSITY PRIOR TO FULL-GENOME SEQUENCING**

ROSE ALBERT & CARLY SNIDOW - UNIVERSITY OF ALABAMA AT BIRMINGHAM

**LYSOGENIC HOST BACTERIUM ALTERS PLATING EFFICIENCY OF GORDONIA BACTERIOPHAGE**

THOMAS HARRINGTON & NOAH THOMPSON - OUACHITA BAPTIST UNIVERSITY

**A STUDY OF PHAGE WITH ATTITUDES: DEFENSIVE GORDONIA PHAGE SIDIOUS AND MAGICMAN AND CRAZY RHODOCOCCLUS PHAGE WHACK AND SLEEPYHEAD**

JESSICA HAYDEN & ANDREW FOURNIER - UNIVERSITY OF MAINE, HONORS COLLEGE

3:00 PM – 3:15 PM	BREAK	LOBBY
3:15 PM – 5:15 PM	POSTER SESSION II   EVEN-NUMBERED POSTERS	LOBBY
5:15 PM – 6:15 PM	DINNER	DINING ROOM
6:15 PM – 6:30 PM	GROUP PHOTO	FRONT ENTRANCE
6:45 PM – 8:00 PM	KEYNOTE II Dr. Graham Hatfull University of Pittsburgh	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
8:00 PM – 9:30 PM	Social	LOBBY
9:00 PM – 11:00 PM	SHUTTLES TO NATIONAL CONFERENCE CENTER (NCC) – EVERY 30 MINS	DRIVEWAY LOOP ENTRANCE

*The shuttle service is for guests that are staying at the NCC.*

# 11<sup>TH</sup> SEA-PHAGES SYMPOSIUM

Sunday, June 9, 2019

7:00 AM – 7:30 AM CHECK-OUT  
*NCC guests will be shuttled from the NCC to Janelia. All guests must check out of their rooms before breakfast. Luggage may be stored in the Gallery and Synapse Meeting Room.*

7:00 AM – 8:15 AM BREAKFAST DINING ROOM

8:30 AM – 10:00 AM ORAL PRESENTATIONS III STUDENTS IN AUDITORIUM  
MODERATOR: MATTHEW MASTROPAOLO FACULTY IN SEMINAR ROOM

## **IDENTIFICATION AND IMPLICATIONS OF SOIL-DWELLING BACTERIAL DNA METHYLTRANSFERASE HOMOLOGS IN MYCOBACTERIUM PHAGE PHALM**

CHRISTINA SPENCER & KATELYN GUTIERREZ - LE TOURNEAU UNIVERSITY

## **CHARACTERIZATION OF A NEW MICROBACTERIUM FOLIORUM CLUSTER EB PHAGE 'STROMBOLI': A TALE OF TOXINS, GIANT LYSINS, HNH ENDONUCLEASES, AND A GENE CLUSTER PREDICTED TO REGULATE NUCLEOTIDE LEVELS.**

STEPHANIE PREISING & AMELIA HOYT - SOUTHERN CONNECTICUT STATE UNIVERSITY

## **COMPARATIVE GENOMIC ANALYSIS OF 31 SIPHOVIRIDAE REVEALS EXTENSIVE HOST-DEPENDENT RELATIONSHIPS AND NOVEL PHAGE SUBCLUSTERS**

ANDREW KAPINOS - UNIVERSITY OF CALIFORNIA, LOS ANGELES

## **COMPARATIVE GENOMICS OF PHAGES ISOLATED ON NEW HOST SPECIES REVEALS NOVEL GENOME FEATURES**

KAITLYN FIELDS & SHAWNA LARSON - UNIVERSITY OF WISCONSIN-RIVER FALLS

## **NON-MYCOBACTERIAL ACTINOBACTERIOPHAGES PROVIDING MORE INSIGHT TO PHAGE BIOLOGY**

ANGELA DE JESUS & MARIANA MORAES - NYACK COLLEGE

## **FUNCTIONAL ANALYSIS OF CONSERVED HYPOTHETICAL GENES IN THE CLUSTER K BACTERIOPHAGE HAMMY**

SAVANNAH UNDERWOOD - UNIVERSITY OF SOUTHERN MISSISSIPPI

10:00 AM – 10:30 AM BREAK LOBBY

10:30 AM – 11:45 AM KEYNOTE III STUDENTS IN AUDITORIUM  
Dr. Steffanie Strathdee FACULTY IN SEMINAR ROOM  
University of California San Diego

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.*



# 11<sup>th</sup> SEA Symposium

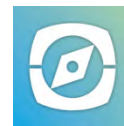
June 7 – 9, 2019

## GENERAL INFORMATION

### PROGRAM BOOK & AGENDA ACCESS

The program book is completely online, and accessible using the mobile app (recommended) or web browser.

1. For access via the mobile app,
  - follow the instructions provided in the email from CrowdCompass,
  - if you have not received the email, download and install the CrowdCompass AttendeeHub app from the **App Store** (iOS devices) or **Google Play Store** (android devices)
  - Once installed, search for “sea-phages”, then login using your name and email address.
2. For access via a web browser, visit <https://event.crowdcompass.com/seasymp2019> and login using your name and email address.



**Note:** Hard copies of the program book and agenda will **NOT** be available at the symposium.

A downloadable/printable agenda (PDF) is also available at the program website at <https://seaphages.org/meetings/45/>.

### STREAMING

All talks in the Auditorium will be streamed live, and can be viewed by anyone via the “LIVE STREAM” button at [www.seaphages.org](http://www.seaphages.org). Feel free to encourage your friends and family to tune in.



### PRESENTERS & MODERATORS | TALKS

Those selected to give talks must upload their presentations to the "SEA Symposium 2019 Talks" folder in Dropbox **by Thursday June 6th, 2019**. 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 from the file you uploaded to Dropbox.

Slides for Session Moderators should similarly be placed in the Dropbox Folder, using the filename as indicated.

As some fraction of attendees will be seated in an overflow room, we recommended that you avoid the use of laser pointers during your talk, as it cannot be seen in the overflow room.

### 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.

## MEETING ROOMS & SEATING ASSIGNMENTS

All talks will be presented in the Auditorium. Talks will also be projected in the Seminar Room, which is also equipped with microphones and video capabilities to ask questions or make comments during the talks.

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

## ATTIRE

Attire for the entire SEA Symposium is business casual.

## MEALS

All meals will be provided at Janelia Research Campus. Dining tables will be reserved for Cohort 12 faculty and Phage Discovery Workshop facilitators for lunch on Saturday.

## 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
- National Conference Center (NCC) – 18980 Upper Belmont Pl, Leesburg, VA 20176 – 703-724-5111

## TRAVEL

- If you have not received your travel and housing confirmations from HHMI Travel Services, you will need to contact [hhmimeetings@hhmi.org](mailto:hhmimeetings@hhmi.org) to request a copy of your travel itinerary.
- Before travelling, please double-check your arrival station/airport and the shuttle schedule. Note that shuttle service is ONLY provided for arrivals into Dulles Airport. Throughout the meeting, shuttle service will be provided between the National Conference Center and the Janelia Research Campus (symposium venue). Guests arriving from other locations will need to arrange for their own ground transportation to Janelia.

## 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/45/>, 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 National Conference Center must leave their cars at the hotel and use the shuttle busses provided from the hotel 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.

## HASHTAG

#seasymp2019



## SYMPOSIUM & HOTEL LOCATIONS

### SYMPOSIUM

HHMI Janelia Research Campus

19700 Helix Drive

Ashburn, VA 20147

Google Map: <https://goo.gl/maps/aQ85zWqKUYWoQ9tx8>

*Tel. 571-209-4000*

<http://www.hhmi.org/janelia>

### HOTEL LOCATIONS

#### **Janelia Research Campus (Janelia)**

19700 Helix Dr,

Ashburn, VA, 20147

Google Map: <https://goo.gl/maps/aQ85zWqKUYWoQ9tx8>

*Tel. 571-209-4000*

#### **National Conference Center (NCC)**

18980 Upper Belmont Pl,

Leesburg, VA 20176

Google Map: <https://goo.gl/maps/fJaBYFupxSDHgiko8>

*Tel. 703-724-5111*

# 11<sup>th</sup> SEA Symposium

## SHUTTLE INFORMATION

Reston Limousine will operate shuttle buses between Dulles Airport, Janelia, and the National Conference Center following the schedule below.

Guests arriving at Dulles Airport proceed to Baggage Claim Area, Carousel #3. A Reston Limousine representative will be holding a sign for HHMI and will direct guests to buses going to Janelia or the National Conference Center.

### ARRIVALS – Friday, June 7, 2019

#### SHUTTLES – Dulles to Janelia and Dulles to National Conference Center\*

Depart at:

12:00 PM

1:15 PM

2:30 PM

#### SHUTTLES – Dulles to Janelia only

Depart at:

3:45 PM

4:15 PM

5:00 PM

**\* Guests arriving at the airport after the departure of the 2:30 PM shuttle should shuttle directly to Janelia. National Conference Center guests can store luggage at Janelia and bring it on the shuttle to the National Conference Center after the evening social activities.)**

#### SHUTTLES – National Conference Center to Janelia

Depart at:

1:30 PM

2:30 PM

3:30 PM

4:30 PM

Guests staying at the National Conference Center can also use the non-HHMI National Conference Center shuttle from Dulles to the National Conference Center.

### EVENINGS – Friday and Saturday

#### SHUTTLES – Janelia to National Conference Center

Depart at:

9:00 PM

9:30 PM

10:00 PM

10:30 PM

11:00 PM

### MORNINGS – Saturday and Sunday

#### SHUTTLES – National Conference Center to Janelia

Depart at:

6:45 AM

7:15 AM

7:45 AM

8:10 AM

### DEPARTURES – Sunday, June 9, 2019

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

Depart at 1:00 PM

#### SHUTTLE – From Janelia to HHMI HQ (for SEA Bioinformatics Meeting Guests Only).

Depart at 2:00 PM

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

<b>SEA Institution</b>	<b>Poster #</b>
Baylor University	Poster #049
Bowling Green State University	Poster #076
Brigham Young University	Poster #110
Cabrini University	Poster #122
Calvin College	Poster #005
Carthage College	Poster #117
Coastal Carolina University	Poster #010
College of Idaho	Poster #053
College of Southern Nevada	Poster #025
College of St. Scholastica	Poster #034
College of William & Mary	Poster #077
Collin College	Poster #009
Columbia State Community College	Poster #101
Culver-Stockton College	Poster #102
Del Mar College	Poster #011
Doane University	Poster #037
Dominican College of Blauvelt	Poster #061
Drexel University	Poster #062
Durham Technical Community College	Poster #051
Fayetteville State University	Poster #050
Florida International University	Poster #017
George Mason University	Poster #115
Gonzaga University	Poster #028
Hampden-Sydney College	Poster #016
Hillsborough Community College	Poster #029
Hope College-Joe Stukey	Poster #035
Hope College-Matthew Gross	Poster #086
Howard Hughes Medical Institute	Poster #045
Howard University	Poster #096
Illinois Wesleyan University	Poster #015
Indian River State College	Poster #112
Indiana University of Pennsylvania	Poster #087
Iowa State University	Poster #064
James Madison University-Tiyam Assadapour	Poster #023
James Madison University-Elise M Rasmussen	Poster #085
James Madison University-Louise Temple	Poster #123
Johns Hopkins University	Poster #075
Kansas State University	Poster #042
La Salle University	Poster #091
La Sierra University	Poster #116
Lafayette College	Poster #109
Lehigh University	Poster #030
LeTourneau University	Poster #002
Massey University	Poster #074
Merrimack College	Poster #033
Miami University	Poster #107
Minnesota State University Moorhead	Poster #097
Mitchell Community College	Poster #007
Monmouth College	Poster #068
Montana Tech of the University of Montana	Poster #078
Montclair State University	Poster #089
Morehouse College	Poster #066
Mount Saint Mary College	Poster #114

Nebraska Wesleyan University	Poster #013
Neumann University	Poster #012
North Carolina A&T State University	Poster #014
Northern State University	Poster #027
Northwest Indian College	Poster #111
Northwestern College	Poster #054
Nyack College	Poster #090
Ouachita Baptist University	Poster #058
Providence College	Poster #070
Purdue University	Poster #120
Queens University of Charlotte	Poster #080
Queensborough Community College	Poster #065
Radford University	Poster #072
Rockland Community College	Poster #057
Saint Joseph's University	Poster #113
Saint Leo University	Poster #079
Salem State University	Poster #105
Southern Connecticut State University	Poster #038
Southern Maine Community College	Poster #044
SUNY Old Westbury	Poster #063
Tarleton State University	Poster #019
The Evergreen State College	Poster #043
The Ohio State University	Poster #046
Truckee Meadows Community College	Poster #082
Universidad Autónoma de Nuevo León	Poster #073
University of Alabama at Birmingham	Poster #055
University of California, Los Angeles	Poster #099
University of California, San Diego	Poster #118
University of Central Oklahoma	Poster #100
University of Colorado at Boulder	Poster #098
University of Connecticut	Poster #081
University of Detroit Mercy	Poster #092
University of Evansville	Poster #032
University of Hawaii at Manoa	Poster #108
University of Ibadan	Poster #024
University of Kansas	Poster #026
University of Lagos	Poster #041
University of Louisiana at Monroe	Poster #022
University of Maine, Farmington	Poster #039
University of Maine, Honors College	Poster #067
University of Mary	Poster #047
University of Mary Washington	Poster #071
University of Maryland, Baltimore County	Poster #094
University of Nebraska-Lincoln	Poster #104
University of Nevada Las Vegas	Poster #048
University of North Georgia	Poster #004
University of North Texas	Poster #059
University of Pittsburgh	Poster #121
University of Puerto Rico at Cayey	Poster #106
University of South Florida-Richard Pollenz	Poster #020
University of South Florida-Louis Otero	Poster #084
University of Southern Mississippi	Poster #093
University of Texas at El Paso	Poster #001
University of the Ozarks	Poster #103

University of the Sciences in Philadelphia	Poster #006
University of West Alabama	Poster #069
University of West Florida	Poster #031
University of Wisconsin-River Falls	Poster #040
Virginia Commonwealth University	Poster #003
Virginia State University	Poster #052
Virginia Tech-Stephanie Voshell	Poster #088
Virginia Tech- Elizabeth Burrell	Poster #095
Virginia Western Community College	Poster #021
Washington University in St. Louis- Nitán Shalon	Poster #018
Washington University in St. Louis- Christopher D Shaffer	Poster #060
Western Carolina University	Poster #119
Western Kentucky University	Poster #083
Winthrop University	Poster #008
Worcester Polytechnic Institute	Poster #036
Xavier University of Louisiana	Poster #056

# 11th Annual SEA Symposium Abstract

Baylor University

Waco TX

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



**Gabriel Andino**

## ***Discovering genetic diversity with NapoleonB***

**Gabriel Andino**, Kathryn Adkins, Sriram Avirneni, Emily Balint, Henry Burns, Lucy Fisher, Lauren Foley, Emily Gaw, Lily Goodman, Soo-Un Jeong, Cooper Johnson, Melissa Leon Norena, Michael Lum, Rachel Melone, Nathan Newton, Aman Patel, Sabin Patel, Lucy Potts, Brandon Reider, Shepard Saaybe, Sona Subramanian, Joseph Yu, Justin Yu, Lathan Lucas, Leo Rule, Aadil Sheikh

*Arthrobacter*, a type of bacteria commonly found in soil and sewage, has recently been used as a host for phage isolation by the SEA-PHAGES program. For this project, *Arthrobacter sp.* ATCC KY3901 was used to isolate *Arthrobacter* phage NapoleonB. This phage was used to explore genetic diversity of AM phages. NapoleonB was isolated using enriched media, and a DNA sample was sequenced by the Pittsburgh Bacteriophage Institute using Illumina sequencing. Genome, annotations were manually curated using tools such as DNA Master, NCBI databases, PhagesDB, HHpred, and Phamerator. After isolation and genome annotation, several questions regarding the uniqueness of NapoleonB and AM phages were raised in the form of %GC content, protein structure and mechanisms, unique repeats, and the potential for super-clustering. To test these questions and further explore NapoleonB's genome, multiple sequence alignments were performed, phamerator maps were analyzed to determine any patterns in synteny, and NapoleonB's holin and endolysin proteins were characterized through tertiary structure predicting computer programs such as Jmol and RaptorX. NapoleonB exhibits siphoviridae morphology and produces two distinct sizes of clear plaques with average diameters of 1.5 mm or 0.1 mm. Bioinformatic annotations indicated 100 potential genes, 73 with no known function and 25 with predicted functions within the 57,846 base pair genome. NapoleonB and other AM phages differ from other *Arthrobacter* phage clusters with significantly lower %GC. It was also noted that all phages in the cluster AM contain a putative holin protein that has previously been annotated as having no known function. Further examination of *Arthrobacter* phage lysin cassettes identified different types of conserved catalytic regions. This information provides examples of what makes NapoleonB and other AM phages unique among other clusters; it helps expand previous knowledge about phage diversity. Future bioinformatic work can address variations in %GC and potential super-clusters using models of horizontal gene transfer and comparative genomics.



# 11th Annual SEA Symposium Abstract

Bowling Green State University

Bowling Green OH

Corresponding Faculty Member: Jill Zeilstra (jill.zeilstra@gmail.com)



**Linnéa Forbes**

## ***The Bowling Green State University Expanding Mosaic of Mycobacterium Bacteriophage***

**Linnéa Forbes**, Rachel Bowling, Benjamin Caskey, Colton Flaherty, Chloe Koon, Edward Madden, Morgan Nance, Lauren Tucker, Ashley Wong, Sayantan R Choudhury, Raymond Larsen, Vipaporn Phuntumart, Zhaohui Xu, Jill Zeilstra-Ryalls

During the fall semester of the 2018-2019 Bowling Green State University SEA-PHAGES Program, 17 bacteriophage were isolated. Among these, one was individually sequenced while DNA from 12 others were combined and sequenced en masse. The sequencing results revealed that the individually sequenced lytic Siphoviridae phage JoieB belongs to the S cluster. This phage was annotated during the spring semester, and a number of characteristics of the genes present in the phage were investigated. The en masse sequencing results for the 12 additional phage revealed that clusters A1, A4, B1, C1, G1, J, and S are represented among them. We were able to determine that the S cluster phage is Pringar. Interestingly, plaque size varied significantly between the two S cluster phage; JoieB formed plaques that were approximately 1 mm in diameter while those of Pringar resembled pinpricks that were too small to measure. Other relationships between the genomes of Joieb and Pringar are discussed. Bowling Green State University has now isolated 41 new bacteriophages, and sequence results to date reveal that they include representatives of the rare S and W clusters.

# 11th Annual SEA Symposium Abstract

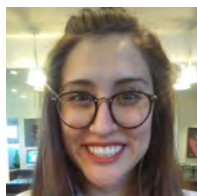
Brigham Young University

Provo UT

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



**Emilee L Carr**



**Rochelle Gaertner**

## ***Isolation of Mycobacteriophages from Sewage***

**Emilee L Carr**, Elise Melhado, Emily Loerscher, Trever Thurgood, Ruchira Sharma, Donald P Breakwell, **Rochelle Gaertner**, Julianne H Grose

Each year there are 2 million reported antibiotic resistant infections and millions more that go on unreported in less developed countries. Alternatives to antibiotics that still effectively lyse and kill bacteria are being researched with the forerunner being bacteriophage therapy. The HHMI SEA Phages program has isolated thousands of phages that infect *Mycobacterium smegmatis*, which can cause edema, redness, and pain in infected soft tissue. In the lab of Doctor Julianne H Grose, we attempted to isolate bacteriophages against *Mycobacterium smegmatis* that are more clinically relevant by using sewage as the isolation source. To characterize these phages, they were isolated from primary effluent from sewage plants, sequenced through high throughput sequencing, and annotated using both the automated system DNA Master and by analyzing coding potential. Ultimately, these phages will fall into phage families whose relativity is based on highly conserved proteins. These proteins may reveal unique characteristics in the phages that allow them to be more or less useful in phage therapy. The *M. smegmatis* phages did fall into pre existing families and the next step is to look at the proteins that are different between the families and the implications thereof. This research will add to the growing understanding of phages which allow the phages to be more fully characterized and therefore, more likely to be used in phage therapy.

# 11th Annual SEA Symposium Abstract

Cabrini University

Radnor PA

Corresponding Faculty Member: Vinayak Mathur (vm7027@cabrini.edu)



**Rya Scull**

## ***Discovery and Annotation of Cluster AN and AK Arthrobacter Phages***

**Rya Scull**, Jessica Azzarano, Jordan Abelson, Jessica Baranoski, Lavinia Harrison, Megan M Wojcik, Sonia Spadafora, Kyriaki Gerasimidis, Olivia Mancini, Epoh Fonge, Isabella Romani, Alyson Marshall, Andrew Conboy, Matthew D Mastropaolo\*, Vinayak Mathur, Melinda Harrison

\* Neumann University, Aston PA

Bacteriophages are viruses that infect a bacteria host, potentially leading to strategies for treating, preventing, or diagnosing bacterial infections such as tuberculosis that are resistant to conventional antibiotics. We have discovered novel phages from the bacterial host *Arthrobacter* sp. ATCC 21022. The phages were collected from soil and water samples by students from various places around South Eastern PA. and Southern NJ. and their genomic DNA isolated. After isolating the genomic DNA, they were photographed through the use of an electron microscope and then the DNA was sequenced. The phages' genomes were then annotated using various bioinformatics tools, such as DNA Master; Phamerator; HHPred and GeneMark to determine gene location and function. Using comparative genomics, unique characteristics of the phage's genome were also explored. This study presents a comparison of several bacteriophage genomes specifically from the AN and AK cluster. The genomes of phages Arby and Scuttle were annotated as a collaborative effort by many students and faculty.

# 11th Annual SEA Symposium Abstract

Calvin College

Grand Rapids MI

Corresponding Faculty Member: John Wertz (jwertz59@calvin.edu)



Emily A Sall

## ***The Puritan and the Phloozy: Genomic insights into the lifestyles of *Gordonia* phages Jabberwocky and Schwartz33***

Noah P Ambrose, Elizabeth C Bolton, Samuel L Braak, Erin S Brink, Anna L Christiansen, Sarah G Gibes, Liam P Hoogewerf, Fanny J Johns, Leah H Knorr, Katherine M Koning, Stephanie L Robinson, Christina D Romano, **Emily A Sall**, Emily G Schellenboom, Lauren M Steffen, William M Terpstra, Nathan J Wilkes, Mary Rose Horner-Richardson, Randall J DeJong, John T Wertz

Six *Gordonia* phages with siphoviridae morphology were isolated from soils in Grand Rapids, Michigan, using *Gordonia terrae* 3612 as a host. Three were isolated via direct plating and three via enrichment. We obtained complete genome sequences from two, Jabberwocky (enrichment; 85 ORF's; subcluster DE1) and Schwartz33 (direct; 89 ORF's; cluster DJ). Jabberwocky is most closely related to Stultis (93.2% nucleotide identity) and Schwartz33 is most closely related to OhMyWard (68.4% nucleotide identity). Jabberwocky's genome contained all of the expected ORF's, but the genome of Schwartz33 was missing several, including tail assembly chaperones and scaffolding proteins. The genome of Schwartz33 also contained nineteen orphans, significantly more than others in this cluster (OhMyWard has six orphans). Several of the orphans were found to have a function, including a capsid maturation protease, a deoxycytidylate deaminase, a ThyX thymidylate synthase, a DNA helicase, and two membrane proteins. This is the second year in a row our phages grouped within DE1 and DJ, so we compared Jabberwocky and Schwartz33 with our previously isolated phages Kroos and Tanis. Jabberwocky and Kroos have moderate synteny but are only 76.3% identical at the nucleotide level. Schwartz33 and Tanis also have moderate synteny but are only 62.4% identical at the nucleotide level. We also used information on phamdb.org to explore possible correlations of clusters DE1 and DJ with geographic location, soil type, and isolation date. No significant correlations were found. Jabberwocky had 37 phams unique to the DE1 subcluster. Of the remaining, most were clusters exclusive to *Gordonia* phages. This suggests that Jabberwocky's genome has evolved from phages with a limited host range. In contrast, Schwartz33 contains 30 phams found exclusively in the DJ cluster, but only six phams were found solely in *Gordonia* phages. 30 phams were also found within the two *Rhodococcus* phages in cluster CC: Pepy6 and Poco6, 14 of which were found within the first 1/3 of the genome and are structural in nature. Schwartz33 also shared 20 phams with the BI cluster (*Streptomyces*); 21 phams with *Arthobacter* clusters AM, AU and AW; 18 with EL (*Microbacterium*); and at least one ORF within

*Mycobacterium* clusters K, L and M. Of the phams found in multiple bacterial genera, 16 had AM, AU, AW, BI, CC and EL together in a single pham. This leads us to believe the evolutionary history of Schwartz33 draws on phages with a broad host range. The fact that Schwartz33 shared 30 phams with *Rhodococcus* phages, many identified as having a structural function, makes testing of DJ phages on a *Rhodococcus* host enticing.

# 11th Annual SEA Symposium Abstract

Carthage College  
Kenosha WI

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



**Mason Fanelle**

## ***Discovery and Analysis of Mashley (Cluster EG), Den3 (Cluster EA1) and Velene (Cluster EA1)***

**Mason Fanelle**, Asher Boucher, Ashlynn Edwards, Cristian Hilliard, Letitia Siers, Krysti Vanovenoeke, Andrew Albers, Amy Cooper, Gelene Rivera, Victor Austin, Madeline Perez, Steven Henle, Qinzi Ji, Sheryl Konrad, Deborah Tobiason

During the Fall semester at Carthage College, 58 bacteriophages were isolated using *Microbacterium foliorum* as a host. These phages were purified and characterized, and three of the phages were chosen for DNA sequencing and annotation (Den3, Velene and Mashley). All three annotated phages are lytic and lack an integrase gene. In addition, these phages have siphoviridae morphology with long, flexible, non-contractile tails. After sequencing and annotation, Den3 and Velene were found to belong to cluster EA1 and are very similar to each other. Mashley belongs to cluster EG and has several orphans. Mashley is most closely related to phage Hyperion though the EG cluster is quite diverse, and Mashley appears to contain unique sections. Further analysis of these phages spans topics from doing whole genome comparisons to focusing on specific genes of interest to determine phylogeny and analyzing regions of interest such as repeated DNA sequences. There is relatively little data available thus far on *Microbacterium* phages; therefore, our research will boost our understanding of *Microbacterium* phages, especially those in clusters EA1 and EG.



# 11th Annual SEA Symposium Abstract

Coastal Carolina University  
Conway SC

Corresponding Faculty Member: Daniel Williams (dwilliams@coastal.edu)



Tyler Cutaia

## ***Isolation and Annotation of *Gordonia* Bacteriophages Mayweather and Kenosha***

Tyler Cutaia, BIOL 492 Students, Daniel C Williams

With the goal of exploring bacteriophage diversity, Phage Discovery students at Coastal Carolina University discovered 7 new phages in the fall semester of 2018. The phages, which infect the host bacterium *Gordonia rubripertincta*, were isolated using enriched isolation techniques. Two of these phages, Mayweather and Kenosha, were sequenced and annotated the following semester. Both phages had plaques of various morphologies, but Mayweather's plaques were very clear, suggesting that this is a virulent phage. Mayweather is a CT cluster phage with a 48,382 bp genome and GC content of 60.6%. We verified 75 predicted genes and assigned functions such as terminase and minor tail proteins to 25. There was little support for functions of the remaining 50 genes and interestingly 9 of them are orphans. In contrast, Kenosha is a DJ cluster phage with a longer genome of 60,899 bp and has a GC content of 51.8%. This phage has 92 predicted genes, 8 of which are orphans. We assigned functions to 20 genes that code for common gene products such as HNH endonuclease, lysin A, and holin. Future research from Coastal students will involve working as part of the SEA-GENES project, where wet bench experiments will be performed to assess whether predicted genes have cytotoxic effects on bacterial host cells.

# 11th Annual SEA Symposium Abstract

College of Idaho

Caldwell ID

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



**Kaiden Lee**

## ***Beauty and the Beast: Characterizing Axym and Untangling Jumble***

Kaiden Lee, Megan Rockefeller, Ann P Koga

In the fall semester, our BIO 210 lab isolated several phages from soil on our campus using *Gordonia terrae* as a host. We sent two samples to University of Pittsburgh for sequencing. In the spring semester we annotated the genome of Axym, which belongs to Cluster CT. Like other CT cluster phages, Axym appears to have a lytic life cycle, no tRNA genes, and a split Lysin A gene found on the far-left end of the genome. As we were annotating Axym, we noticed a high level of similarity to a subset of CT phages and very little similarity to other CT phages. Thus, we compared the CT phages using the Gene Content Comparison tool available on Phagesdb and by SplitsTree4 analysis (Huson, 2006). From these data, we propose that Axym, along with five other CT phages, should be placed in a new subcluster within the CT cluster. Our second sample, aptly named Jumble, contained a mixture of 2 genomes: one from the DG cluster (Jumble\_DG) and one from the CQ cluster (Jumble\_CQ). In order to annotate these two genomes, we first needed to separate and purify them for archiving and determine which phage went with which genome sequence. We designed primers for each phage based on unique sequences in their tape measure genes, determined optimal PCR conditions and repeated plaque purification several times in order to get pure cultures. We created lysates, then used the primer sets to test for the presence of each genome in the purified lysates. We also re-examined the electron microscopy images and found phages with two different tail lengths. Using tape measure gene length, we were able to match each EM image to the correct phage and genome.

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# 11th Annual SEA Symposium Abstract

College of Southern Nevada

Las Vegas NV

Corresponding Faculty Member: James Theoret (james.theoret@csn.edu)



D'Andrew L Harrington

## ***The College of Southern Nevada's Exploration and Annotation into the DR and CS Clusters throughout the Las Vegas Valley***

D'Andrew L Harrington

Throughout the 2018-2019 Fall and Spring semesters, the College of Southern Nevada's SEA-PHAGES team studied methods of articulation with biological and computational sciences regarding the extraction, annotation, and experimentation of novel *Gordonia*-phages Jellybones and Nhagos. Both phages were discovered within the Las Vegas valley and were identified using the host bacterium *Gordonia rubripertincta*. While sharing the same morphotype, siphoviridae, both phages provided unique overall results. Jellybones exhibited optimal temperatures differing from Nevada's average soil temperatures.

Jellybones, a CS2 subcluster *Gordonia*-phage, was discovered within the City of Henderson's Bird Viewing Preserve. Producing plaques ranging from ½ - 1mm, Jellybones has a genomic length of 77,514 base pairs covering 108 encoded genes; 1 transfer RNA was detected with an anti-codon of GTT (Asparagine). A GC percentage of 59.0% was above the 58.9% average for this subcluster. 32.0% of Jellybones was annotated without a classification of NKF. Annotations of *Gordonia*-phage Jellybones was completed with; DNA Master, GeneMark, HHPred, Blast, Starterator, Phamerator, SOSUI, and TMHMM. Experimentation of Jellybones was conducted to determine calcium dependency and temperature ranges for optimal lytic cycles. Results of our temperature assay shows an optimal temperature for the lytic cycle at 20 °C. Results of our calcium assay show plaque growth in the presence of calcium through a lytic cycle. These results can be used for future investigation of *Gordonia*-phages by the College of Southern Nevada's cohort.

NHagos, a DR cluster *Gordonia*-phage, was discovered within a flamingo exhibit. Producing small, clear and round plaques, Nhagos is a circularly permuted genome sequence with a genomic length of 59,580 base pairs covering 83 encoded genes; no tRNA was detected. A GC of 68.2%, was lower than the 69.2% average for the DR cluster. Annotation and review through Gepard indicate a similar mosaic structure to that of the B (*Mycobacterium*) and W (*Mycobacterium*) clusters. We replicated these findings by providing three separate phages from each cluster; The W cluster consisted of *Mycobacterium*-phages Cepens, GodPhather, and Megabear, while, The B cluster consisted of *Mycobacterium*-phages Cheet0, Apex, and Abinghost. These

similarities are measurable within Phamerator throughout each cluster, indicating levels of homology within each cluster. This method of determining homology could, in theory, reduce the amount of NKF proteins found relating to morphotype. The College of Southern Nevada is proud to share its first findings for the SEA-PHAGES symposium and how temperature, calcium, and mosaic patterns could be found in a sea of collaboration.

# 11th Annual SEA Symposium Abstract

College of St. Scholastica  
Duluth MN

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

## ***Phylogenetic and genomic characterization of Actinobacteriophages NadineRae and PetterN***

**Ryan J Steger**, Lauren E Buchholz, Breanna D Cole, Kelsey K Fletcher, Ashlie K Johnson, Robin N Kutsi, Chloe S Larson, Nathan A Schacht, Anna C Totsch, Daniel E Westholm

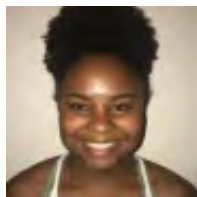
Actinobacteriophages NadineRae and PetterN were isolated on *Gordonia terrae* and *Mycobacterium smegmatis*, respectively, as part of the SEA-PHAGES program at The College of Saint Scholastica. Genomic sequencing of NadineRae revealed a 64,714 bp genome with 66.1% GC content and subcluster CR4 designation. MEGA Phylogenetic analysis indicated *Gordonia* phage Marietta, isolated in 2017 by a St. Scholastica student, was among the closest relatives to NadineRae. Expanded phylogenetic comparisons with both *Gordonia* phages and *Gordonia* bacterial species did not present a clear relationship between *Gordonia* phage genomes and the particular *Gordonia* species with which they were isolated. Phamerator comparisons with close CR4 relatives indicated several areas of sequence repeats on the right arm of the genome between coordinates 57,000-63,000. When NadineRae's genome was BLASTed against itself, three distinct sequence repeats were mapped to this region. Within these coordinates, the first repeat with consensus sequence GTAGGGCCCGGCGAGATCTCGCGCCGGCCCTAC appeared 12 times, the second with consensus sequence GTAGCTACCCCGTAGGGCCCGGCGAGATCTCGCGC appeared 12 times, and the third with consensus sequence GACGCGCAGCGCGTGC---GCCGACGCGGCC appeared 10 times. All three of these repeats appeared elsewhere in the genome, but at lower frequency. The precise function of these repeats was not fully elucidated, but the DNA motif prediction software TomTom identified possible repressor or activator binding activity. In addition, NadineRae protein expression patterns were examined using tandem mass spectrometry. Liquid infections of NadineRae were performed for 4 hours, pelleted and subjected to LC-MS/MS to identify expressed phage proteins. Data is not yet available, but hopefully will be by the symposium. Finally, the lysogeny related genomic sequences of the temperate Mycobacteriophage PetterN were examined. Using the DNA Scan feature in DNA Master 19 stoperator sequences were identified with consensus sequence GTACGATGTCAAG. Most of the genes where these stoperator sequences occurred had no known function, but several played structural or assembly roles.

# 11th Annual SEA Symposium Abstract

College of William & Mary

Williamsburg VA

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



**Taiana J James**



**Solene M Sossah**

## ***Mycobacteria achiense Phages: Continuing Challenges and Unsolved Puzzles***

**Taiana J James**, Gokul Achayaraj, Oluwatobi W Adepoju, Emin Ahsan, Phillip A Andoh, Hallie F Avalos, Sanjana Challa, Kiara C Douglas, Madison P Foster, Ingrid V Guardado-Cruz, Nicole A Harris, Thomas M Hess, Joshua R Hughes, Soledad V Jimenez, Jerry S Sanchez-Lopez, Daiwik P Munjwani, Elias H Nafziger, Bisola N Olowe, Helen S Owusu, Veda S Pai, Ray Q Smith, **Solene M Sossah**, Angelique T Vo, Yessica A Bonilla, Elizabeth N Do, Alexandra A Huang-Queiroz, Autumn Liu, Camille C Okonkwo, Sudip Paudel, Mark H Forsyth, Margaret S Saha

In an attempt to expand the diversity of phages that infect hosts within the *Mycobacterium* genus, 22 freshmen who comprised the William and Mary 2018-19 SEAPHAGES Lab attempted to identify phages that infected *Mycobacterium achiense*. We selected this host because we wished to investigate host range in *Mycobacterium* species other than *M. smegmatis* given the clinical importance of the genus and also because there are currently no reports of phage isolated from *M. achiense*. Moreover in the past year, high school students from Jamestown, Virginia participating in phage outreach events at William and Mary, isolated over 50 phages from this host. However after eight weeks and several hundred enrichment attempts in the 2018-2019 SEAPHAGES program, only a single phage was isolated – HerbertWM. Working with HerbertWM presented challenges and puzzles at every step of the process. The phage consistently had extremely low titers that declined quickly over time. DNA isolation was equally challenging. Following optimization of DNA extraction methods, PEG precipitation of large volume liquid cultures produced a sufficient amount of DNA for sequencing. Sequencing resulted in a 51 kb phage that may represent a new subcluster within the A cluster. Surprisingly, there was also a 11 kb “phagelet” that shared little identity with the 51 kb HerbertWM, but did encode a terminase, a tape measure protein, and several structural proteins. A current hypothesis proposes that this 11 kb fragment may represent a satellite phage; all phages isolated from this species appear to have



the 11 kb fragment based on gel analysis. Sequencing of the host and other *M. achense* phages is underway to resolve the ongoing puzzle of the unique bacteriophages isolated from this host.

# 11th Annual SEA Symposium Abstract

Collin College

Plano TX

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



**Elizabeth S Hampton**



**Danielle J Davis**

## ***Annotation of Phage McGalleon from Subcluster EA1 in *Microbacterium foliorum****

Elizabeth S Hampton, Danielle J Davis, Thanh-Huy Nguyen, Kirk S Niekamp, Kaitlyn M Riley, Carole M Twichell, Jonathan N Lawson, Bridgette L Kirkpatrick

This project highlights the genome annotation of the siphoviridae bacteriophage McGalleon (MG). McGalleon is a lytic phage isolated using *Microbacterium foliorum*, and is a member of cluster EA, subcluster EA1. McGalleon has a genome length of 42,562 bp, and a 63.70%, currently the longest genome of the EA cluster and the highest GC% of the subcluster. Annotation utilized the bioinformatics tools DNAMaster, Glimmer, Genemark, PhagesDB BLAST, NCBI BLAST, HHPred, and Phamerator. Of the 65 genes identified through auto-annotation, three genes were deleted due to lack of coding potential. Analysis supported assigning functions to 26 of the 62 annotated genes, and two previously unidentified regions were discovered during manual annotation: MG\_2 and MG\_52. MG\_2 is an insertion near the beginning of the genome. MG\_52 is located where two genes were predicted to be, based on their presence in most phage in the subcluster. However, MG\_52 lacks similarity to either of the missing genes normally found in the region surrounding MG\_52. McGalleon is missing a gene predicted to have phosphoesterase activity, which is found in 51 of 64 phage from EA1.

# 11th Annual SEA Symposium Abstract

Columbia State Community College  
Columbia TN

Corresponding Faculty Member: Elvira Eivazova (eeivazova@columbiastate.edu)



**Gregory Markov**

## ***Isolation, characterization and annotation of bacteriophages using the soil-dwelling host *Microbacterium foliorum*.***

**Gregory Markov**, Ximena Leon, Megan Mohundro, Jenna St. Pierre, Carter Woehlert, Tessa Cote, Tristan Watson

Bacteriophages, viruses that specifically infect bacteria, are the most abundant organisms on the planet. Phages have been of interest to scientists because they can efficiently destroy bacteria, and therefore, can be used for phage therapy. We isolated and characterized bacteriophages using a known host, the soil-dwelling bacterium *Microbacterium foliorum*. Bacteriophages were purified and amplified to increase the concentration of phage particles. The isolated bacteriophage genomic DNA was purified, treated with restriction enzymes and characterized using gel electrophoresis. The viral samples were visualized using transmission electron microscopy. We observed various viral life cycles: the lytic life cycle, where phages infect and rapidly kill infected host cells, as well as the lysogenic life cycle, where phages integrate into the host genome, instead of directly killing the host. The purified phage DNA samples were sequenced at Pittsburgh Bacteriophage Institute, and the novel sequences were annotated at CSCC. Phages Manatee and Vanisius are members of the Siphoviridae family. Manatee is a temperate phage belonging to the A cluster, A1 subcluster. Vanisius is a lytic phage belonging to the EE cluster. The genomes are considerably different in size with no gene sequence similarity. The genomes were analyzed based on their start and stop codons, z-scores, identity matches, cross-references in Phamerator, and between NCBI and HHPred toolkits. Our future goal is to further explore phage genome communities isolated from the immediate environment. Evaluating and understanding phage biology and taxonomy is essential to the development of phage therapy approaches and biotechnology application.

# 11th Annual SEA Symposium Abstract

Culver-Stockton College  
Canton MO

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



**Drew N Klocke**

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

Muhammad E Seegulam, Zachary M English, Alexander R Heaney, Julianna N Hollman, Bryan J Hunter, **Drew N Klocke**

In this experiment we sought to isolate and characterize a novel bacteriophage from an environmental sample. A soil sample was collected from the Northeast Missouri region and putative phages amplified using *M. smegmatis* mc2155 as a bacterial host. The sample was subjected to several rounds of purification with the aim of isolating a novel bacteriophage. The presence of bacteriophage was inferred by the formation of clearings, or plaques, on bacterial lawns that had been infected with samples containing the putative phage. Three new phages were discovered by the current cohort: JoeDirt26, SchmutzDaCat, and Pass. Genomic analysis was conducted on bacteriophage Avadakedavra isolated by the 2015 cohort. The Avadakedavra genome was 73721 base pairs long, with cluster L assignment and subcluster L1. Minor tail protein, membrane domain protein, and capsid maturation protease were among the known products encoded by the 121 features identified in a preliminary analysis of the Avadakedavra genome, while 65 were found to have no known function. Also encoded in the Avadakedavra genome is a single tRNA, tRNA-Cys(TGC). The programs used for annotation include DNA Master, Phamerator, Starterator, HHpred, NCBI BLAST and PECAAN.

# 11th Annual SEA Symposium Abstract

Del Mar College  
Corpus Christi TX

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**Alexis S Trujillo**

## ***The Isolation, Characterization and Genomic Annotation of a A16 Bacteriophage ‘Lucyedi’***

Alexis S Trujillo, John Ramirez, Lorie Leyva, John R Hatherill, Daiyuan Zhang

Bacteriophages are viruses that attack and kill their host bacteria. It has been estimated that there are over  $10^{31}$  bacteriophages present on our planet and more and more phages have been used for gene therapy and treatment for antibiotic resistant bacterial infections. In this study, isolation of a novel bacteriophage ‘Lucyedi’ began with a soil enrichment procedure followed by several experiments to characterize the isolated phage using its bacteria host *Mycobacterium smegmatis*. A high titer lysate was harvested for phage genomic DNA isolation. The restriction digest analysis and genomic sequencing were conducted using the isolated DNA. The phage morphology of ‘Lucyedi’ was studied by uranyl acetate negative staining and transmission electron microscope imaging. Lysogens of ‘Lucyedi’ were isolated from spot tests with extra incubation and used for phage efficiency studies on its host. The plaques of ‘Lucyedi’ indicated a lytic life cycle at the time of isolation. The TEM images show ‘Lucyedi’ contains a capsid with 60nm in diameter and a tail 160 nm in length. The restriction digest patterns suggested the ‘Lucyedi’ genome contains multiple recognition sites for BamHI, ClaI, HaeIII and few sites for HindIII and EcoRI. Both the TEM image and restriction pattern imply that ‘Lucyedi’ belongs to the cluster A. The lysogen efficiency test of ‘Lucyedi’ indicated that 99.14% of the host *Mycobacterium smegmatis* could be destroyed by this bacteriophage, which makes ‘Lucyedi’ a potential candidate to develop a phage treatment for pathogenic *Mycobacterium tuberculosis*, a close species to *M. smegmatis*. Lucyedi’s genome has been sequenced by the Pittsburgh Bacteriophage Institute. The dsDNA genome is made up of 92 open reading frames or potential genes, with a genomic length of 52,987 BP and a 61.4% GC content. This particular phage belongs to the Cluster A, Subcluster A16. Cluster A is known as the largest group of Actinobacteriophages with the majority of them being mycobacteriophages. The genome of these phages have defined ends with 3’ overhangs. Currently only two bacteriophages including ‘Lucyedi’ have been identified to belong in Subcluster A16.

# 11th Annual SEA Symposium Abstract

Doane University

Crete NE

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



**Lilly Shatford-Adams**

## ***GENOME ANNOTATION OF MYCOBACTERIOPHAGES DUGGIE, KLOPPINATOR, HOCUS IN THE B1 SUBCLUSTER***

**Lilly Shatford-Adams**, Trevin Alberts, Ayden Benavides, Alexis Burke, Sam Coy, Nick Crespo, Kylie Crnkovich, Salvador Delgadillo, Serenity Kinswoman, Anna Korte, Haley Miller, Brenna Mulvey, Reagan Peterson, Makenna Weddle, Kade Wehrs, Dane M Bowder, Erin L Doyle

Micro-organisms called bacteriophages, or phages, are viruses found in abundance around the world. Mycobacteriophages Duggie and Hocus were discovered in soil samples at Doane University in Crete, Nebraska in September of 2018; Kloppinator was found by Johns Hopkins University in Baltimore, Maryland in 2012. After isolating, purifying, and amplifying the bacteriophages, their structures were examined through Transmission Electron Microscopy (TEM) images. Through this process, it was determined that all three phages are of siphoviridae morphology, meaning they have non-enveloped capsule heads and have long, non-contractile tails. Afterwards, the phages' genomic DNA was extracted and sequenced, establishing that all three are lytic and part of the B1 cluster. The lengths of the genomes range from 68,053 to 68,885 base pairs with approximately 100 predicted genes in each. We used the software DNA Master, Starterator, Phamerator, and BLAST in order to predict the start codons of open reading frames (ORFs) by comparing each feature and genome to the features and genome of similar phage. Using HHpred and NCBI BLAST, we deduced the function of each gene to find its importance in the genome. Most of the functions were found to be unknown which is not uncommon for Mycobacteriophages. The information that we accumulated through our annotation can be used by others in the future when they are predicting the same aspects of their genome.

# 11th Annual SEA Symposium Abstract

Dominican College of Blauvelt  
Orangeburg NY

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

## ***Retrieval of Mycobacteriophage from Different Types of Soils***

**Darlenys Sanchez**

In the current study, mycobacteriophages Penelope2018 (D1) and Guwapp (C1) were collected from compost-enriched soil. Mycobacteriophage are viruses that infect members of the genus *Mycobacterium*. It is advised to search for these bacteriophage in moist soil rich with organic matter. It is hypothesized that phages will be retained in rich soil and not retained in sand or clay. Five soil types were tested, namely, commercial potting soil, clay, compost, peat, and sand. In addition, three types of clay were analyzed. The soils were sterilized and phage were added along with phage isolation buffer and/or host bacterium. The mixtures were incubated at 30°C for 3 and 5 days, after which phage presence was tested using a spot plate procedure. Results indicate that clay does not support phage retention, and the titer of the phage decreased significantly within 5 days. The sand and peat retained phage to the greatest degree, with potting soil and compost to a lower degree. Future studies include an assessment of pH in the ability to retrieve phage from the various soil types, as well as aeration status of the soil, in terms of the ability to retrieve phage from the soil. Annotations of these genomes was completed, and an analysis into the amino acids found in the major tail subunit and capsid proteins will be done. This research informs students isolating and purifying phage that soils such as sand may also harbor mycobacteriophage.

# 11th Annual SEA Symposium Abstract

Drexel University  
Philadelphia PA

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



**Nicholas Barbieri**



**Erin Gallagher**

## ***Novel Bacteriophages Against Environmental Phactors – Phage stability Put to the Test!***

**Nicholas Barbieri, Erin Gallagher, Amal Ahmed, Sanya Ailani, Deeksha Ajeya, Mervin Alexander, Gabriela Almanzar, Shehbeel Arif, Neil Bal, Pooja Balar, Sam Beane, Vincent Beggarly, Sumaiya Begum, Atharva Bidaye, Meg Bindiganavale, Amber Bolli, Nowrin Borsha, Sydney Bucher, Sarah Busby, Steven DeLuca, Chloë Denley, Emmett Dessimoz, Spoorthi Dingari, Capri DiSaverio, Vesa Dobi, Alexander Fahmy, Prachi Gaddam, Ayaan Gill, Dale Glova, Nishunta Gopalasundaram, Virginia Granato, Sayema Hakim, Samuel Hawk, Dakota Heizman, Aaron Jeong, Kailee Johnson, Gianna Joyner, Sammy Khalouf, Chaeyeon Kim, Joyce Kim, Nila Kirupaharan, Hetal Lad, Yong Hui Lin, Vincent Liu, Kaylen Louie, Isabella Mancini, Zachary Mandell, Tracy Marcelis, Bhakti Marfatia, Akos Marfo-Sarbeng, Kathleen Marsili, Ryneisha McKenzie, Derek Mock, Riyaz Mohamed, Assal Nasir, Anh Nguyen Quach, Isabella Pappano, Pallavi Pareek, Nidhi Patel, Prarthana Patel, Shruti Patel, Thara Patel, Arathi Pillai, Anjali Pradhan, Shrey Pradhan, Anjani Ravi, Sophie Redila, Byron Ruf, Mekhala Santebennur, Elana Sargent, Shivashree Sekar, Elisa Selamaj, Simran Shamith, Mekhala Santebennur, Elana Sargent, Shivashree Sekar, Elisa Selamaj, Simran Shamith, Harsh Sharma, Kumal Siddiq, Seena Soroush, Annika Surapaneni, Almas Thaha, Summer Thompson, Amy Varghese, Akhil Vindyala, Pavithra Vinnakota, Haider Virk, Nisha Vora, Lincoln Wu, Merry Xiang, Seth Zurlo, Ritu Dalia, Susan Gurney, Svetlana Khakhina**

In the Fall of 2018, Drexel University students isolated 36 novel bacteriophages using *Microbacterium foliorum* as a host. All phages were isolated using enriched isolation and 8 of them were sequenced. The Illumina sequencing of these phages demonstrated that all phages have lytic cell cycle, siphoviridae morphology and belong to EA1, EA4 subclusters and EC cluster. These phages were successfully annotated using DNA Master, Phamerator, NCBI Blast and PECAAN and submitted to GenBank. During the Spring quarter, we investigated the effect of varying environmental factors on phage stability by evaluating changes in the plaque forming units (pfu) observed. Phages were exposed to acidic or basic pH, high temperature, different glucose concentrations or UV light. Additionally, the protective effect of whey protein concentrate (WPC) and alpha-



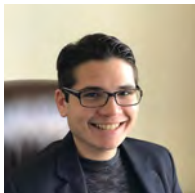
lactalbumin on phage stability, when exposed to high-temperature, was also investigated. Full plate plaque assays were conducted in all the experiments. If phage stability was adversely affected, the number of pfu would decrease. Conversely, an increase in pfu would indicate that the tested condition has enhanced the ability of the phages to infect the bacterial cell. Our results demonstrated that exposing phages to acidic or basic conditions or UV light showed a decrease in pfu counts. Another interesting observation was that exposure to glucose also significantly decreased phage stability, probably due to osmotic shock and phage lysis. Other experiments showed that adding whey protein to phage lysates stabilized the phage during high-temperature shock, suggesting that it has a protective effect which enhanced phage infectivity. Surprisingly, alpha-lactalbumin had an opposite effect on phage stability, when exposed to high temperature, because decreased pfu results were observed when compared to the control plates (using phage buffer alone). Based on these observations we conclude that phages can tolerate exposure to a range of environmental factors and are still able to perform bacterial cell infections.

# 11th Annual SEA Symposium Abstract

Durham Technical Community College

Durham NC

Corresponding Faculty Member: Marie Fogarty (fogartym@durhamtech.edu)



**Kieran H Murthy**

## ***LOST AND PHOUND: Identifying Diverse Phages Using DOGEMS and Annotation of Sixama & MinecraftSteve***

**Kieran H Murthy**, Caleb A Christie, Alimatou S Diallo, Zavier Dixon, Peyton M McIntosh, Maggie G Rosen, Lee M Simpson, Marie P Fogarty

Recently, phage therapy is being reconsidered as a viable alternative to antibiotics. This movement has created a drive to collect, document and annotate as many phages as possible. In fall 2018, the Phage Hunters collected soil samples from the Durham area of NC, resulting in isolation of 16 phages using the host *Gordonia terrae*. Sixama, discovered by direct isolation, was sequenced individually. DNA from ten of the remaining phages was combined and sequenced using the DOGEMS approach. The spring 2019 Phage Hunters annotated Sixama's genome using DNAMaster and PECAAN. Sixama was found to be from the uncommon cluster DS. This made annotation somewhat challenging due to lack of comparative data. Following sequencing and assembling of the DOGEMS sample, a further six complete genomes were identified, along with two mostly complete genomes and one partially complete genome - all from different clusters. To match phage identity to genome sequence, we designed primers specific to each of the nine contig sequences using NCBI Primer-BLAST. Specificity of each primer set for its cluster was confirmed using phagesdb BLAST. Using DNA from each phage sample, PCR was carried out for all nine contigs. The ideal and expected result was that only one of the ten DNA samples would amplify for each contig tested. Using this approach, seven out of the ten phages were successfully matched with their genomes. MinecraftSteve, a subcluster A15 phage, was subsequently selected for annotation. 167 genes were annotated for Sixama and 98 genes for MinecraftSteve. While only three tRNAs were annotated in MinecraftSteve, 29 were annotated in Sixama. Interestingly, Sixama has a GC content of 52.7%, quite different from that of *Gordonia terrae* (67.8%), while MinecraftSteve has a GC content of 62.0%. The excess of tRNAs encoded by Sixama may help compensate for the compositional differences between the phage and host genomes.<sup>1</sup> Both annotated phages are temperate, but only Sixama has an integrase gene. Instead, MinecraftSteve encodes parA and parB, which likely allows the prophage to form a stable plasmid within the host cell<sup>2</sup>. Five possible sites were identified where Sixama may integrate into its host genome. Three of the potential integration sites overlap tRNA genes within the host, a common location where temperate phages integrate into bacterial genomes. Additionally in MinecraftSteve, a translational frameshift was annotated in the tail assembly chaperone genes. In conclusion, using the DOGEMS approach we demonstrated that collecting phages from various locations and environments may yield a more diverse range of phages within the classroom. Phages were isolated from nine different clusters - two phage genomes

were fully annotated and six further phage genome sequences identified through DOGEMS are available for annotation.

1. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1987346/>
2. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4998052/>

# 11th Annual SEA Symposium Abstract

The Evergreen State College  
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Ashley Walker

## ***A BLAST Result from the Past: Isolation and Analysis of Archived Cluster A6 Phage Hexamo***

Ashley Walker, Tessa Jacobs, Emily Smith, Alberto Napuli, James Neitzel

The student participants in the 2014-15 year of the SEA--PHAGE project were 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. During this cycle 23 phages were isolated from local soils, purified, and entered into the PhagesDB collection. During fall 2017, during a DOGEMS (Deconvolution of Genomes after En Mass Sequencing) run a new A6 phage was assembled and PCR experiments identified this phage as the archived Hexamo from this collection. As relatively few A6 genomes had been completely annotated at that point, we used the supplied total sequence to fully annotate the genome of this phage.

This set of phages had their DNA purified and analyzed by restriction enzyme digestion and gel electrophoresis. Hexamo was visualized by transmission electron microscopy after negative staining with uranyl acetate. This resulted in a clear image which appeared to be *siphoviridae*. DNA from these phages was sequenced using the Illumina process at the Pittsburg Bacteriophage Institute. The sequence of Hexamo revealed a 52,359 bp linear double stranded DNA genome with a sticky ten bp 3' overhang and with a GC content of 61.4 %. Analysis of the sequence of this phage confirmed that it was a siphoviridae in the A6 subcluster. BLASTn results indicated its closest relative was Artemis2UCLA. The genome was 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 using Phamerator. Predicted starts were also examined using Starterator. Preliminary results suggest the presence of 105 protein coding genes and a predicted translational frame shift. Particular care was taken in the region around the minor tail proteins and head-tail connector region to use best available data to annotate this region. All expected genes for a temperate replication mode were present. We identified 3 tRNA genes using ARAGORN and tRNAscan-SE. The final annotation passed quality control and is now in GenBank as accession MK 359360.

# 11th Annual SEA Symposium Abstract

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**Karina Gonzalez**

## ***Phylogenetic and protein structure comparison of minor tail proteins among F cluster phages***

Daniel Cambron, Ana Ruas, **Karina Gonzalez**

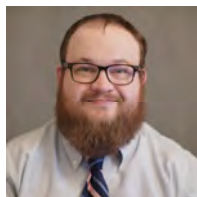
The annotation of phage genomes commonly results in the annotation of multiple minor tail proteins per genome, without distinguishing whether there are distinctive differences or functions of these multiple minor tail proteins. While research into the evolution of long-tailed phages has focused on bench work and functional annotation, comparisons of the protein structure of the multiple minor tail proteins harbored by these phages are lacking. We identified 78 minor tail proteins present in ten phages from the F cluster representing subclusters F1, F2, F3, and F4. To visualize the evolutionary relationship among these minor tail proteins, we constructed a maximum likelihood phylogenetic tree using PROMALS3D and IQ-TREE. We then identified distinct clades of minor tail proteins from the phylogenetic tree and predicted the protein structure for one representative of each clade using the program SWISS-MODEL. Similarities in 3D structure between proteins within the same clade and across clades were examined. By examining how these proteins group in the phylogenetic analysis and whether they cluster according to the sub-cluster they belong to or if they cluster across different phages, we will develop a greater understanding of the diversity and conservation of minor tail proteins and their structures. Future research in this area should focus on examining the structural conservation of minor tail proteins across phages of different clusters and, ultimately, different hosts.

# 11th Annual SEA Symposium Abstract

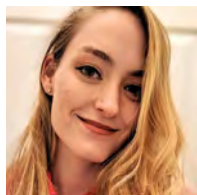
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**John Perkins**



**Lauren M Pincus**

## ***Identification of two *Gordonia* phages: SteamedHams and Suerte***

Alena Arlova, Dion M Blackmon, Sianna Burnett, Dominic E Cruz, Deleram Dastan, Juan D Esteban-Lopez, Jorge D Fernandez Davilla, Sara Hull, Alex Irizarry, Lulu Lwin, Anam Naz, **John Perkins**, **Lauren M Pincus**, Najiyah A Prome, Shah Saeed, Nicola Solares, Annie H Tran, Tina Zhen, Wanyue Zou, Mohamad A Alayouni, Anne E Scherer, Bonnie Madden, Brian Nguyen

Here we describe two novel bacteriophages, SteamedHams and Suerte, isolated in Northern Virginia via the enrichment method using *Gordonia rubripertincta* NRRL B-16540 as the host. Both genomes were sequenced using Illumina sequencing at the Pittsburgh Bacteriophage Institute.

SteamedHams (named for a famous Albany delicacy) is a lytic phage that forms small clear plaques and is a cluster CT *Gordonia* phage. The genome is 44,571 bp in length and has a GC content of 59.9%. It was isolated from a soil sample taken near a community garden in Alexandria, VA. SteamedHams has 63 predicted gene products.

Suerte (“lucky” in Spanish) is a cluster CZ *Gordonia* phage that was isolated from a busy sidewalk on the George Mason University campus in Fairfax, VA. Suerte forms small clear plaques, though cluster CZ phages are typically temperate. The genome is 47,306 bp long and has GC content of 66.5%. Suerte has 76 predicted gene products including genes consistent with a temperate lifestyle such as a tyrosine integrase. We have also identified the common core sequence used to integrate into the host genome during lysogeny. Suerte has a potential translational frameshift in the tail assembly chaperone. To better identify the slippery site, we developed software, coded in Python, to help characterize the site of the translational frameshift in the tail assembly chaperone gene by searching for conserved slippery sequences across the CZ cluster.

# 11th Annual SEA Symposium Abstract

Gonzaga University

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Ian Melder

## ***A jumbo discovery: Omphalos is the largest actinobacteriophage found to date***

Jared DeBruin, Eleanor Johnson, Matthew McCaw, Sarah Vetsch, **Ian Melder**, Meghan Casey, Kate Delaney, Grace Garza, Christina Holland, Darby Howat, Martina Hunt, Logan James, Caroline Kotson, Rika Linman, Angela Palant, Skylar Riley, Olivia Roty, Spring 2019 Genetics Lab students, Kirk Anders, Ann-Scott Ettinger, William Ettinger, Marta Fay, Marianne Poxleitner, Sean McKenzie

To expand knowledge of bacteriophage diversity in the phylum Actinobacteria, we searched for phages that could grow on the host *Microbacterium testaceum* NRRL B-59317. *M. testaceum* isolates typically live on and in plants without causing disease symptoms. *M. testaceum* NRRL B-59317, however, was found in a NASA clean room during assembly of the Mars lander, Phoenix. Phage Omphalos was isolated from soil sampled from a pumpkin garden and enriched in medium containing *M. testaceum*. Omphalos produced large, turbid plaques at 30 deg C with an irregular, multi-ringed appearance. Clear plaques routinely appeared on these plates, but we have not propagated them to test heritability. At 37 deg C, all plaques were clear. TEM showed that Omphalos is a non-contractile tailed phage (capsid width, 100 nm; tail length, 250 nm). The Omphalos genome is 235,841 bp in length, which places it in the group of >200 kb phages known as giant or jumbo phages. It is the first jumbo phage discovered in the Actinobacteria. The genome ends contain long, direct terminal repeats of 18,632 bp. The GC content of Omphalos is 34%, whereas the *M. testaceum* genome is 70% GC. Omphalos contains 318 predicted protein-coding genes, 33 of which are duplicated in the direct terminal repeats, and two tRNA genes. The genome is a singleton among actinobacteriophages, but is distantly related to a clade of extrachromosomal DNAs in bacterial genomes which are annotated as large plasmids, as well as the singleton *Bacillus* phage bp0305Phi836 and the singleton *Acinetobacter* phage vB-AbaM-ME3. In total we identified 37 supposedly bacterial contigs or plasmids related to Omphalos, all of which were approximately the size of jumbo phage genomes (150-800 kb) and contained terminase, portal, and capsid proteins. Most of these were found in the genomes of species in the phylum Firmicutes, a phylum distantly related to the Actinobacteria which has convergently evolved a gram-positive cell wall morphology. The DNA sequences most closely related to Omphalos all came from bacteria in the genus *Bacillus*, and shared on average 127 genes with Omphalos (as determined by blastp similarity). Many shared genes are syntenic between Omphalos and the *Bacillus* sequences. Regions of 80-90 kb containing structural genes encoding terminase, portal, major capsid protein, and tail proteins are in nearly identical order. The strongest alignments show 35-75% amino

acid identity across the length of the proteins. The major capsid proteins, for example, share 71% aa identity. To the right of this region, genes with functions related to DNA replication, DNA metabolism, and recombination are shared between Omphalos and the *Bacillus* sequences, but gene order is not as well conserved.



# 11th Annual SEA Symposium Abstract

Hampden-Sydney College

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**Damian Martinez Pineda**

## ***Phinding Phages a Good Home: A Comparative Genomic Survey of 7 adopted Cluster A Mycobacterium smegmatis phages***

**Damian Martinez Pineda**, David Z Bushhouse, Blake A Martin, Austin C Murphy, Harrison R Whaley, Zachary P Wiggan, Corey J Williams, Michael J Wolyniak

While considerable work has been done by SEA-PHAGES and other groups to characterize *Mycobacterium smegmatis* cluster A phages, a large number of isolated cluster A phages remain uncharacterized and could potentially add invaluable insights to bacteriophage evolution and lifestyle. In this spirit, we adopted 7 cluster A *M. smegmatis* bacteriophages from SEA-PHAGES (Rutherford (A1), Whabigail7 (A2), Veracruz (A3), Bumblebee11 (A4), Scorpia (A5), Jordennis (A6), Expelliarmus (A8)) and did a comparative genomic annotation of the group. As shown by previous SEA-PHAGES work, all 7 phages displayed similar sizes and overall genomic architecture despite their discoveries from disparate locations across the United States. Despite these overall similarities, our study also revealed unique features in individual or subgroups of the 7 phages. Among these features were a 430 basepair gap in the Veracruz genome revealed by BLAST to represent a transfer event from the *M. smegmatis* genome, immunity repressor proteins in similar locations near the back ends of the Expelliarmus and Bumblebee11 genomes, and a section of the Rutherford genome that defied the traditional front-half/back-half opposing direction of ORFs traditionally found in this cluster. Taken together, this study reinforces what has been previously revealed about the genomic architecture of *M. smegmatis* cluster A phages while revealing potentially intriguing individual adaptations that individual phages have made across different geographical locations.

# 11th Annual SEA Symposium Abstract

Hillsborough Community College

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**Michael Alpert**

## ***Genomic Analysis of Microbacterium Phage HarperAnne***

**Michael Alpert**, Nathalia Assaad, Ginelle Bonnelly, Serina Bush, Brandi Fafarak, Courtez Greene, Joseph Murphy, Chau Phan, Leo Rea, Margaret Hopson-Fernandes, Christine Logue, Lisa Smith, David Wingfield, Jim Wysong

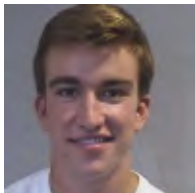
Antibiotic resistant bacterial infections are projected to be the leading cause of death by the year 2050. Bacteriophages, viruses that exclusively infect bacterial cells, are a promising solution to this pending health crisis. Phages are the most abundant biological particle on the planet. They are evolutionarily ancient and genetically highly diverse. Studying phage genomes gives valuable insight into the proteins coded for in phage DNA that allow them to infect their host and avoid their hosts' defenses. HarperAnne was discovered during the wet lab portion of the course in an organic soil sample collected in Pinellas County on September 11, 2018. This lytic phage infects the host *Microbacterium foliorum* NRRL B-24224 SEA, a nonpathogenic relative of infectious bacteria including *Mycobacterium abscessus*. Phage DNA was extracted using HHMI SEA-PHAGES protocols. Sequencing, completed by the Pittsburgh Bacteriophage Institute, revealed that HarperAnne is a Cluster EE phage with a genome length of 17,116 bp, 26 genes, a 9 bp 3' sticky overhang, and 68.8% GC content. BLAST results indicated the closest annotated phage relatives are Noelani and Miaurora. The purpose of this study was to compare the genome of HarperAnne to phages in the same pham to identify similar and unique gene products. We hypothesized that there would be synteny among structural and assembly function genes and that proteins with the same function would have regions of high amino acid identity. We compared HarperAnne to phages in the pham using several different predictive programs including Phamerator and HHPred. We found a high degree of similarity of structural and assembly function genes. Structural genes identified include terminase, portal protein, major capsid protein, head-to-tail connector complex protein, minor tail protein, tail assembly chaperone, and lysin A. There is one orpham. No tRNAs are present. Our results indicate a high degree of similarity in gene products between HarperAnne and other phages from the same cluster. Our findings will contribute additional information to the relatively limited body of knowledge about the diversity within and between phages that infect different Actinobacterial hosts.

# 11th Annual SEA Symposium Abstract

Hope College

Holland MI

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**Matthew Gross**

## ***Mycobacteriophages Paphu and Philly: Two New Members of Two Familiar Clusters***

**Matthew Gross**, Megan Lopez, Emma Beemer, Isabelle Bertolone, Kayla Brady, Mikayla Coombs, Sara Filippelli, Jessica Liu, Morgan Malaga, Anna Molloy, Shane McAuthur, Jonathan Outen, Victoria Parker, Carleigh Robinson, Hannah Tegtmeyer, Mikayla Zobeck

Sixteen 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. A variety of plaque morphologies were produced based on size, shape, and clarity; both lytic and temperate phages appear represented in this collection. The mycobacteriophages, Paphu and Philly, were chosen as two of three phages for complete genome sequencing and comparative genomic analyses. The predominant plaque produced by Paphu after 48 hours at 32°C was circular and was approximately 2-3 mm in diameter. The plaque morphology was clear with a turbid ring resulting in a halo visual. The predominant plaque produced by Philly after 48 hours at 32°C was circular, clear, and was approximately 1 mm in diameter. The complete genome sequence for Paphu revealed a relationship to mycobacteriophages of cluster A, subcluster A1, which now contains 154 sequenced members. Paphu is most similar to the A1 phages AFIS and Blue. The genome size of Paphu is 51,159 bp, which is smaller than most of the sequenced A1 mycobacteriophages. However, it has one of the highest GC contents of A1 phages at 64.1%. The Paphu genome contains 92 protein-encoding genes and no tRNA or tmRNA genes. Despite the genomic identities organizing phages into subcluster A1, they show marked levels of genomic diversity, scattered throughout their genomes. The complete genome sequence for Philly revealed a relationship to mycobacteriophages of cluster B, subcluster B3, which now contains 31 sequenced members. Interestingly, all but a few B3 phages have been isolated between Michigan and Washington, D.C. The genome of Philly is 68,523 bp, which is smaller than most of the sequenced B3 mycobacteriophages. It has a GC content of 67.5%, similar to other B3 phages as well as the host *M. smegmatis* (67.4%). The Philly genome contains 102 protein-encoding genes and no tRNA or tmRNA genes. Phages in subcluster B3 share very high sequence identity throughout the genome lengths. Philly maintains this pattern, being nearly identical to mycobacteriophages Heathcliff, Athena, and Bernado despite their isolation in different years (2003-present) and in different geographical locations. However, Philly does contain some genomic variability around genes 2, 5, and 56. And like all B3 phages, Philly also contains many short sequence repeats throughout its genome.

# 11th Annual SEA Symposium Abstract

Hope College

Holland MI

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**Joseph Stukey**

## ***6° of Separation: The Impact of Temperature on Isolation of Cluster K Mycobacteriophages***

Bethany Van Houten, Geordan Stukey, Adam Krahn, Angela Vito, Gloria Chang, Frank Moen, Adam Slater, Aaron Best, **Joseph Stukey**

Mycobacteriophages are viruses that infect mycobacterial hosts. Over 1750 mycobacteriophages are organized into 39 distinct clusters based on genetic similarity. Some cluster A and K mycobacteriophages can also infect *Mycobacterium tuberculosis*, a distinction of potential medical importance. Hope College SEA-PHAGES students have been isolating possible cluster K mycobacteriophages at a higher frequency ( $\approx 2x$ ) after lowering the isolation temperature from 37°C to about 32°C (30°-32°C). These 32°C-isolated phages were unable to propagate at 42°C. PCR analysis supported cluster K classification for many possible cluster K phages isolated at 32°C (23 of 30 tested of a total of 43), but few isolated at 37°C (3 out of a total of 23). All 3 PCR-supported K phages isolated at 37°C grew at 42°C. We have sequenced 16 of the possible cluster K phages, and found all PCR-supported phages, including the 3 isolated at 37°C, belong to cluster K. We hypothesize that the observed higher Cluster K phage isolation frequency is at least partly due to a relative growth advantage at lower temperatures that is fully compromised at 42°C. Results from experiments testing specific growth parameters, including phage thermostability, adsorption rate, latent period, and burst size, are consistent with our hypothesis. In an effort to determine the step of the lytic cycle blocked at 42°C, a temperature down-shift experiment (42°C shifted down to 32°C) was performed on Hyperbowlee, a cluster K phage isolated at 32°C and growth inhibited at 42°C. Assays were performed testing two different 42°C hold time lengths. Both assays produced a consistent shift in time to first release of new Hyperbowlee phage, to a point about 85 minutes following the temperature down-shift. These results and subsequent tests indicated that phage infection was blocked, post DNA transfer, at about 20-30 minutes into the 32°C lytic cycle. Additional investigations into phage stability under drying conditions and competition growth assays are underway.

# 11th Annual SEA Symposium Abstract

Howard University

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## ***A Kaleidoscope of phage Clusters***

Kedus . Ashagre , Amia . Black, Laraine Cheung, Diana Effiom, Folasade Fashina , Sanaa Haamen, Ramata Haidara , Amber A Johnson, Esther Nwozo , Delandra Robinson, Triniti Sims, D'nai N Thomas, Students of HU- PHAGES 2018-2019, Glory Bassey, Victoria Brooks, Michelle L Fernando, Madison Moore, Jerome Oliver, Benedict Quagraine , Swagota Roy , Michael Smith, Adrian A Allen, Mary A Ayuk, Ayele Gugssa, Courtney J Robinson, Ghosh Somiranjan, Hemayet Ullah , Anderson Winston , . .

Mycobacteriophages are viruses that infect mycobacterial hosts, including *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. These are studied for purposes which include viral ecology, epidemiology, tools in molecular biology and in the development of therapeutics. The aim of this project was to explore phage diversity through isolation and characterization of unique mycobacteriophages from 125 soil samples collected from the Howard university environment, using *Mycobacterium smegmatis* as the host. Almost all the phages were derived from the enrichment culture.

Subsequently, phages were purified using standard protocols, lysates generated, DNA was isolated, quantitated, characterized and representative samples sequenced by the Pittsburgh Bacteriophage Institute, using Illumina Sequencing. Sequenced genomes are currently being annotated, (except Delton which is already approved with GenBank Accession MK559427), using various in-silico programs. With Dallas and Jonghyun identified as temperate, their lysogens have been used to characterize other phages. Furthermore, all phages are being utilized for identifying patterns of phage sensitivity/insensitivity—immunity testing using wildtype *M. smegmatis* mc2155 and generate lysogens.

Data analysis indicate that sequenced phages can be divided into five clusters: B (phage SynergyX, Abinghost and Bananafish; C (Blackbrain, Cactojaque, Kboogie, Trinitium and YoungMoneymata ) G1 (Jonghyun ), J (Dallas) and a singleton (Onyinye ). GC content ranged from 64.7- 68.9% and was identified for morphotypes Siphoviridae and Myoviridae. In addition, preliminary data from sensitivity/insensitivity testing suggests that several of these phages are lytic.

Availability of the genomic information from such diverse clusters will help the study of bacteriophage diversity and their evolutionary mechanisms that give rise to the vast diversity seen in the bacteriophages."

# 11th Annual SEA Symposium Abstract

Illinois Wesleyan University  
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**Katie Vogler**



**Jackson Rapala**

## ***A Phamily of Phages: Sucha, Warren, and Celaena***

**Katie Vogler, Jackson Rapala,** Emmet Agting, Vanessa Chapa, Matt Crosse, Ivy Do, Maeghan Eaker, Anjali Nimavat, Manish Pathuri, Kylie Spiegel, Saralex Torres, Lane Warren, Saylor Williams, Richard Alvey, David Bollivar

Bacteriophages are the most numerous known biological entities on Earth yet relatively few have been cultured and even fewer have had their genomes sequenced and analyzed. Throughout the 2018-2019 school year students at Illinois Wesleyan University were able to discover 16 unique bacteriophages that infect the host *Microbacterium foliorum* NRRL B-24224 SEA. From these, three were selected to be sequenced and functions were assigned for their identified genes using PECAAN and DNA Master. These phages were chosen because they showed the greatest probability of being unique based on laboratory experiments including lysogen testing, polymerase chain reaction (PCR) with primers designed to detect the most common *M. foliorum* phage clusters, and because of their interesting plaque morphologies and transmission electron micrographs. Lysogeny did not occur for any of these phages and the absence of integrase genes in the genomes supports this observation, suggesting they are virulent with a lytic lifestyle. The three *Siphoviridae* phages sequenced were Sucha, Warren, and Celaena. Warren and Sucha were paired with singletons to form the new clusters GA and EJ respectively. Celaena is a new member of the EB cluster. Despite being in different clusters, annotation of Sucha and Warren showed that these two phages shared many highly similar predicted proteins with many genes being in the same pham. In a Splitstree analysis of the sequenced *M. foliorum* phages, these EJ and GA clusters appear to be closely related but are quite distant from Celaena.

# 11th Annual SEA Symposium Abstract

Indian River State College

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**Denisse L Hasan**

## ***Individual and en masse sequencing of 10 Actinobacteriophage from 7 clusters and one singleton***

Denisse L Hasan, Helen Wiersma-Koch, Tom D'Elia

Prior to the 2018-2019 academic year, Indian River State College successfully isolated 50 Actinobacteriophage from soil samples as a part of the HHMI SEA-PHAGES program. Of the isolated phages, 16 have been sequenced with a total of 9 phages being classified as belonging to cluster A. The other phage are classified into 7 different clusters. In order to isolate a broader range of phage outside of cluster A, the IRSC program has implemented a new strategy leading to the sequencing of 10 Actinobacteriophage genomes in 2018-2019. As a result, the overall phage diversity at IRSC has expanded, with a total of 26 genomes sequenced representing 11 clusters and one singleton. Three bacteriophage were selected for individual sequencing. Two of these phage, Yeet (J) and Lolavinca (C1), belong to clusters not previously isolated at IRSC. The third individually sequenced phage, Hulk, is the second EE cluster microbacteriophage isolated at IRSC. DNA available from the remaining 7 mycobacteriophages was pooled together for en masse sequencing in the DOGEMS project. Through this process, the genomes of the most unique phage are able to be assembled for annotation. This sequencing strategy successfully yielded 7 individual genomes. The singleton IdentityCrisis was the most unique phage sequenced, having the smallest genome size of any mycobacteriophage. The remaining 6 genomes were from bacteriophage that belong to clusters or subclusters not previously identified at IRSC (IdentityCrisis, singleton; Cintron, A4; Magpie, B; Mangeria, C; Harella, E; Yeet, J; Whitty, T). These findings also add new phage to clusters that are still relatively low in abundance, including clusters T and J, which have only 7 and 37 phage, respectively. Utilizing the DOGEMS strategy for genome sequencing revealed that soil in Southeast Florida is very diverse in mycobacteriophage. As a result, a novel singleton has been sequenced and additional phage have been added to clusters T and J.

## 11th Annual SEA Symposium Abstract

Indiana University of Pennsylvania

Indiana PA

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Claire Shemon

### ***Beaglebox: a Mycobacterium smegmatis phage isolated from a dog kennel near IUP***

Claire Shemon, Jacob King, Kaitlyn Murphy, Hayley Kepple, Brandon Vought, Seema Bharathan, Carl Luciano, Cuong Diep

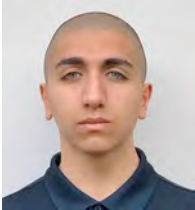
Beaglebox is a B1 subcluster lytic *M. smegmatis* phage that was isolated at a former dog kennel near the campus of Indiana University of Pennsylvania (isolated by K. Murphy, H. Kepple, B. Vought in 2017). It has a Siphoviridae morphotype and creates small clear plaques with fuzzy edges. The genome consists of 68,418 bp with a 66.5% GC content, coding for 103 genes predicted by auto-annotation. During our annotation, we deleted 5 genes (gp3, 10, 45, 78, 81) due to lack of BLAST matches and poor or no coding potential. We also added 2 new genes between large gaps that had BLAST matches and coding potential. The first added gene was between gp11-12 and the second was between gp69-70. Beagle also had one orpham with 468 bp (gp58). Our final annotation showed that Beaglebox contained 100 total genes (all coding for proteins) with 49% of them being assigned functions. Although a holin gene is yet to be identified in this subcluster, we found that our gp16 partially matched holin in the *Corynebacterium* phage Juicebox (38% identity and  $E=1e-10$ ). Further experimentation will be needed to confirm whether this is a true holin gene or not.



# 11th Annual SEA Symposium Abstract

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**Tiyam Assadapour**

## ***Redefining “Multiplicity of Infection”: Two Hosts and Two Phages in One Class***

**Tiyam Assadapour**, Jawad Ahmed, Simon Anderson, Kurt Espinosa, Trniti Gadsden, Allison Graham, William Hajjar, Trent Howard, Olivia Lacafta, Katelyn Matney, Katie Matsen, Johnathan Osu, Easton Rupe, Hong Sang, SoeYu Wadi, Jackson McNeal

With the advent of antibiotic resistance, alternative methods of treatment, such as phage therapy, are being investigated in order to lessen global dependence on antibiotics. The discovery of novel bacteriophages is imperative to designing efficacious phage treatments. Undergraduates involved in JMU’s Viral Discovery courses propagated phages on *Pseudomonas putida*, a gram negative aerobic soil bacterium involved in industrial production and bioremediation. *P. putida* serves as a harmless surrogate for *P. aeruginosa*, a significant pathogen of patients with cystic fibrosis and immunocompromised burn victims. *P. aeruginosa* is known to be highly resistant to multiple classes of antibiotics and thus is an ideal target for phage therapy. Zuri, a siphovirus, was isolated on *P. putida* from garden soil collected in Harrisonburg, VA.

Early in the semester, we discovered that a bacterium similar in morphology and color to *P. putida* was lurking as a “contaminant” in our bacterial cultures. After isolating a pure culture and performing 16s r-DNA sequencing, we identified this bacterium as an uncharacterized species of Microbacterium and tentatively designated it *M. ISAT203*. Microbacterium is a gram positive aerobic soil bacterium, commonly used in commercial cheese production, that causes infections in plants and rarely in humans. We isolated several phages on the Microbacterium host, and Phage lamGroot, found in garden soil from Damascus, MD, was chosen for genome sequencing and analysis. Isolation and purification of the bacteriophages was performed according to standard SEA-PHAGES protocols; however, a simplified alternative DNA purification procedure was implemented. Phages were sequenced at North Carolina State University Genomic Sciences Laboratory (Zuri) and the Pittsburgh Bacteriophage Institute (lamGroot). The ~1 million raw data reads were randomly cut to 125,000 reads, then assembled using Newbler into a single contig for each phage, with >50x coverage. Sequence analysis and annotation were performed according to SEA-PHAGES methodology, and Phage Term (Galaxy) was used to confirm the ends and DNA packaging method.

Zuri is a terminally redundant and circularly permuted phage containing a 75,853 base pair (bp) genome with a 53.5% GC content. One hundred protein coding genes and 3 tRNA genes were predicted. Using BLAST, Zuri was

found to be distantly related to Pseudomonas phages Inbricus and phiAxp-3 (~30% coverage). lamGroot has a 45,625 bp genome containing 74 protein coding genes with a 70% GC content. Fixed ends are predicted with 457 bp direct repeats and an 11 bp 3' overhang. lamGroot is somewhat related to Microbacterium phages Percival and Floof (65% coverage). There is an unusual 300 bp gap in lamGroot, but the region lacks any obvious coding potential. Both phages are temperate, given the presence of recombinase and other related genes.

# 11th Annual SEA Symposium Abstract

James Madison University

Harrisonburg VA

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



**Elise M Rasmussen**

## ***A Novel Approach to Improving Automated Bacteriophage Genome Annotation Utilizing Machine Learning***

Elise M Rasmussen, Steven G Cresawn

Genome annotation tools such as Glimmer and GeneMark use sophisticated mathematical techniques to model the characteristics of genes, however the quality of these models is fixed from the point at which they are created. They fail to adapt to newly available genome sequences or the refinements in annotations provided by expert human reviewers.

In contrast machine learning utilizes algorithms and statistical modeling to solve problems by relying on learned patterns. It has emerging applications in numerous fields including bioinformatics. Machine learning can be supervised or unsupervised. In supervised machine learning a subject area expert guides the algorithm to the appropriate conclusions. Supervised machine learning is divided into two major processes: regression and classification. Regression is used to predict a continuous output from a given input. Classification predicts the category the data belongs to based on the provided input parameters. It is utilized for predicting discrete responses and was selected over regression as the more appropriate method for gene prediction. After developing a model to predict genes, the model was then compared to expert human-generated annotations or those produced by hidden Markov modeling-based approaches such as Glimmer or GeneMark.

A neural network was created using the TensorFlow machine learning toolkit and the Python programming language. Input parameters for the model included gene length, direction, direction of upstream and downstream genes, distance to the preceding upstream stop codon, and a frequency table of dinucleotides within the coding sequence. The model was trained using nearly all SEA-PHAGES quality-controlled, protein-coding genes from phages that infect Actinobacteria.

# 11th Annual SEA Symposium Abstract

James Madison University

Harrisonburg VA

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



**Louise Temple**

## ***Give Them An Inch And They'll Take A Mile: Creative Phage Projects***

**Louise Temple, Stephanie Stockwell**

Calling all phage groupies! Want to play a phage board game? Work a puzzle? Listen to rap or song with unique lyrics? Read a children's book? Stand in awe of amazing poetry? Just ask your students to create something—anything creative, really—about their own experiences with discovery and analysis of phages. “I could never think of anything,” they often say, when I announce this assignment. And yet, semester after semester, clever, unique, surprising contributions are presented to classmate and added to my growing collection of Phantastic Phun Phage Art. A number of items will be displayed at the symposium for your enjoyment and amazement.

# 11th Annual SEA Symposium Abstract

Johns Hopkins University  
Baltimore MD

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



**Sruthi Katakam**

## ***Microbacteriophages ChickenKing and Benjalauren share synteny but low homology in the the EA cluster***

Sruthi Katakam, Lucas Polack, Benjamin Fry, JHU PhageHunters, Emily J Fisher

PhagesDB currently lists 1474 phages isolated on *M. foliorum*, 177 of which have been sequenced. Of those, 88 are in the EA cluster. This year, students at Johns Hopkins University discovered and annotated the genome of ChickenKing, a siphoviridae that defines a new subcluster, EA9. The most closely-related genome is that of Schubert, a cluster EA8 phage discovered in 2017 at the University of Pittsburgh. Though other EA genomes are not identical in terms of sequence homology, they share most genes and 41/57 genes in ChickenKing are shared in the EA1 genome of Benjalauren, another phage isolated at JHU this year. Like other EA cluster phages, ChickenKing and Benjalauren are lytic and their genomes lack an integrase gene. They also both have two-part tail assembly chaperone genes and we were unable to identify a programmed frameshift that would unite them into one gene. We present comparisons of EA1, EA8, and EA9 genomes and discuss approaches to calling genes in a new subcluster in which Starterator and Phamerator sometimes lack information.

# 11th Annual SEA Symposium Abstract

Kansas State University  
Manhattan KS

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

## ***Flint Hills Phages - Characterization of Microbacterium foliorum cluster EB & Gordonia terrae clusters CZ, CV, and subcluster CY1 Bacteriophages***

**Jordan R Block**, Andrew M Braun, Jacob M Darsow, Nollan G Dillavou, Benjamin L Engle, Isaac D Fitz, Samuel E Forbes, Shay E Gear, Isaac M Gutsch, Megan N Johnson, Camryn D Lopez, Tony F Ngassi, Paige E Poolman, Camryn G Splichal, Arendje Takata, Drake A Vanbuhler, Lauren M West, Megan A Willmon, Martha Smith Caldas, Christopher D Herren

For three years, Kansas State University has been isolating mycobacteriophages from enriched soil samples in *Mycobacterium smegmatis*. This year's project expanded our host range to include *Microbacterium foliorum* NRRL B-24224 SEA and *Gordonia terrae* CAG3. Our first semester yielded a 100% isolation rate, with 22 students isolating two *M. foliorum* and 20 *G. terrae* phages. Of the four genomes sequenced and annotated, one was a *M. foliorum* phage and three were *G. terrae* phages. BubbaBear, an EB cluster *M. foliorum* phage, is a small (69 genes), lytic phage that replicates with a particularly large plaque morphology. Included in its relatively small genome are 5 genes associated with thymidine metabolism, including thymidylate kinase, thymidylate synthase, dUTP pyrophosphatase, dihydrofolate reductase and thioredoxin. Four of the five genes in this pathway are found in all 18 EB cluster members, while the 5th gene, dUTP pyrophosphatase, is found in 17 of the 18 members. EnalisNailo is an average-sized temperate CY1 subcluster *G. terrae* phage. It has 66 genes, including two putative Y-integrase genes adjacent to each other in the genome. Five other of the eight CY1 subcluster phages also have this double, adjacent Y-integrase gene arrangement. Temperate phages customarily have a single integrase gene to establish lysogeny, so the conserved presence of two complete integrase genes adjacent to each other, but in separate pham groups in the CY1 subcluster, is of interest. Faith5x5 is a temperate *G. terrae* phage in the CZ cluster, but does not classify further into any of the seven known subclusters. This cluster and its subcluster are sparsely populated with mostly draft genomes. The CZ cluster, subclusters excluded, has only two members and the members are very divergent in gene content on both arms of the genome. Unlike some members of the cluster, Faith5x5 has a single holin A gene instead of a 2-gene holin A coding arrangement seen in other CZ cluster genomes. Wocket is a CV cluster temperate *G. terrae* phage with 79 genes. Included in the genome are two lysin A genes corresponding to the amidase and C39 peptidase domains. While proven to be temperate by lysogeny assays, excise was not able to be identified in the genome, while S-integrase, immunity repressor, and a CRO/HTH DNA binding domain were putatively identified by sequence analyses.

# 11th Annual SEA Symposium Abstract

La Salle University

Philadelphia PA

Corresponding Faculty Member: Jason Diaz (diaz@lasalle.edu)



**Mary C Yan**

## ***Investigating the Programmed Frameshift in Buttons Gene 22***

Mary C Yan, Sean McClory, Jason Diaz

In ISBT 104, my class annotated our adopted mycobacterium phage, Buttons. The Mycobacterium phage Buttons was discovered by Christina Jodway in Milbridge, Maine as part of the SEA-PHAGES program. This particular phage was isolated from the bacterial species Mycobacterium smegmatis and its genome was sequenced. Buttons is classified in the subcluster A1. This phage genome length is 49,420 base pairs long. There are 86 genes in Buttons. The predicted life cycle for Buttons is the lysogenic life cycle. Buttons has a “slippery” sequence in gene 22 where the ribosome makes a mistake and changes the reading frame during translation. This causes the ribosome to make a longer protein in Buttons where the first half is approximately aligned to the short-form of the gene but the rest of the sequence is different. Programmed translational frameshifting (PTF) is an alternate process in protein translation. PTF usually happens in the tail-assembly chaperone proteins. The tail-assembly chaperone protein comes in two forms of the small subunit and the large subunit. In genes 22 and 23 of Buttons, gene 22 encodes the small subunit of the tail-assembly chaperone protein, and PTF causes fusion of genes 22 and 23 to create the large subunit. PTF is one way phages can regulate protein levels without the use of a new promoter. Overall, PTF helps the phage maintain the correct proportion of chaperone proteins to efficiently assemble the tail fiber for Buttons.

# 11th Annual SEA Symposium Abstract

La Sierra University

Riverside CA

Corresponding Faculty Member: Natasha Dean (ndean@lasierra.edu)



**Uylae Kim**

## ***Isolation and analysis of two *Gordonia* phages, CherryonLim and OhMyWard***

**Uylae Kim**, Bryn Batin, Emily Choi, Jasleen Dharni, Sydney Figueroa, Sunil Kim, Loren Klim, Yun Seok Lee, Diana Lim, Axel Nathaniel, Chung Chun Shih, Kristen Simental, Elizabeth Suh, Roshni Trivedi, Isabela Valladolid, Corrine Wang, Cassandra Ward, Keina Yoo, Jessica Choi, Natasha Dean, Arun Muthiah, Arturo Diaz

Students from La Sierra University isolated nineteen bacteriophages from several locations near Riverside, CA either through enrichment culture or direct plating. Fifteen of the phages were isolated from *Microbacterium foliorum*, and four from *Gordonia rubripertincta*.

Host range assays were performed on seven bacteriophages based on the results of preliminary experiments. The efficiency of plating was calculated for four related species of *Microbacterium* and two species of *Gordonia*. Plaquing efficiencies at different growth temperatures (25°C, 30°C, and 37°C) were also measured for each phage.

We selected two lytic *Gordonia* phages, CherryonLim and OhMyWard, for further investigation. Morphological analysis showed that both phages belong to the family *Siphoviridae*, which is characterized by a non-enveloped head, double-stranded DNA, and a non-contractile tail. Illumina sequencing was used to assign CherryonLim and OhMyWard into clusters CT and DJ, respectively. DNA Master and PECAAN were used to annotate each genome by assigning gene functions to putative ORFs.

CherryonLim is 48,948 base pairs long with 72 genes, making it the largest phage in its cluster. It also has a GC-content of 60.2%, and a 3' single-strand extension of 13 bases. Phages in cluster CT are not known to have tRNAs and this is true for CherryonLim. OhMyWard has a genome length of 60,978 base pairs with 85 genes that are all in the forward direction, a GC-content of 52.2% and a 3' single-strand extension of 9 bases. It is similar to other phages in cluster DJ, but interestingly it has the greatest number of orphans.



# 11th Annual SEA Symposium Abstract

Lafayette College

Easton PA

Corresponding Faculty Member: Bob Kurt (kurtr@lafayette.edu)



**Maria Padilla**

## ***PauloDiaboli from Lafayette College: Examining Genes 170, Ribonucleotide reductase R2-1 small subunit, and 174 ,Ribonucleotide reductase 2.***

**Maria Padilla, Abigail Esposito, Austin Best, Manuel Ospina-Giraldo, Robert Kurt**

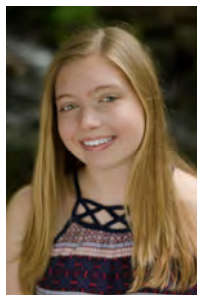
Microbacterium phage, PauloDiaboli, was discovered by utilizing the method of direct isolation. PauloDiaboli was found from a soil sample collected at Lafayette College, Easton PA. The idea for the name arose from the tiny plaques the phage consistently generated, leading to multiple titrations until appropriate-sized plaques were displayed. There was a point in which we thought the phage stock was no longer viable, and due to the phage's stubbornness, the Latin meaning of "little devil" inspired us to name him PauloDiaboli. With a genome composed of 191968 base pairs and with a GC content of 60.09%, we decided to examine PauloDiaboli's genes 170 and 174. Uniquely enough, PauloDiaboli was identified as a singleton phage. However, we were able to find similarities among gene function in PauloDiaboli's genetic makeup. Setting a focus on protein folding led us to place our attention on the influence that alpha helices and beta strands have in the protein sequence that contributes to the genetic makeup of PauloDiaboli. Utilizing UnitProt and HHPred, we studied the specific roles of the active site and metal binding site of both of these structures in PauloDiaboli. While the active site aids in generating GDP, essential for energy supply, the presence of the metal binding site facilitates mineral absorption by the host. How is protein folding such an essential aspect to consider in a singleton phage? Since acquiring and storing energy is facilitated in phages, we concluded that the concentration of both of these protein structures are required to enable phages similar to PauloDiaboli to thrive in diverse environments.

# 11th Annual SEA Symposium Abstract

Lehigh University

Bethlehem PA

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



Caitlin Murphy



Grace Ciabattoni

## ***Exploring a SEA of Phages: New Insights into Phage Genome Diversity, Host-Phage Interactions, and Immunity Relationships***

Caitlin Murphy, Grace Ciabattoni, Nicholas DesGranges, Longhui Gao, Brianna Gipson, Katherine Volpe, Matthew Green, Marika Livingston, Journey Lopez, Hansen Lukman, Ryan Matthiessen, Paige McCloskey, Brielle Paul, Hannah Schuster, Jamie Korenberg, Netta Cudkevich, Catherine Mageeney, Hamidu Mohammed, Margaret Kenna, Vassie Ware

Lehigh's SEA-PHAGES program provides a continuum of collaborative research opportunities for first year and advanced undergraduates who focus on isolating and characterizing Actinobacter phages to gain a better understanding of phage genome structural diversity, gene function, and phage biology in general. Additionally, our group has investigated immunity mechanisms that protect Cluster N lysogens from attack by heterotypic phages. Prophage-mediated immunity, conferred by Cluster N *Mycobacterium* phage Butters, has been the primary focus of our studies to understand mechanisms that differentially protect a Butters lysogen from infection by Cluster A3 phage PurpleHaze and Cluster I1 phage Island3. Here we report on progress on several projects. **I.** In addition to uncovering 12 new phages infecting *Mycobacterium smegmatis* including two new Cluster N phages, Smurph and ShrimpFriedEgg, we tested all direct samples on an additional host, *Microbacterium natoriense*. *Microbacterium* phage Theresita was isolated. **II.** An estimate of cluster diversity was obtained from DOGEMS analysis and three new mycobacteriophage genomes were annotated (two Cluster N phages [Smurph, ShrimpFriedEgg] and Cluster A11 phage Orange). Students also focused on testing infectivity of newly isolated *M. smegmatis* phages on Cluster N Xeno lysogen lawns, and discovered one Cluster A11 phage (Orange) with reduced infectivity compared to infectivity on *M. smegmatis* lawns. Immunity

experiments between Cluster N and A11 phages isolated from past Xeno experiments (Bud, Bowtie, Munch) continue. **III.** Annotation of *Microbacterium* phage Theresita (Subcluster EA7) revealed a chimeric genome organization with left arm nucleotide conservation with Cluster EA phages and right arm nucleotide similarity with Cluster EJ phages. **IV.** We have previously shown that Butters gp30 is required for defense against PurpleHaze infection. This requirement for mediating defense against PurpleHaze infection was further supported by comparative immunity analyses using several *M. smegmatis* strains, lacking or expressing gp30. To investigate the mechanism of Butters prophage-mediated defense, defense escape mutants (DEMs) for PurpleHaze and Island3 were isolated and genomes sequenced. PurpleHaze DEMS mutations mapped to genes encoding minor tail proteins. Interestingly, recovery and sequencing of Island3 DEMS revealed the presence of hybrid phages generated by recombination between Butters and Island3 phage genomes where significant homology exists. Lysogens from hybrid phages, referred to as BIB phages (**B**utters-**I**sland3-**B**utters), are currently being generated for additional immunity studies. Collectively, this array of investigations highlights ongoing research undertaken by Lehigh's SEA-PHAGES students to broaden our understanding of phage-host interactions and phage gene expression.

# 11th Annual SEA Symposium Abstract

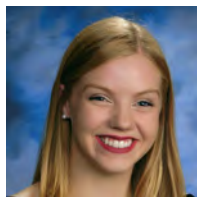
LeTourneau University

Longview TX

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



**Christina E Spencer**



**Katelyn Gutierrez**

## ***Identification and Implications of Soil-Dwelling Bacterial DNA Methyltransferase homologs in Mycobacterium Phage Phalm***

Christina E Spencer, Katelyn Gutierrez, Frederick N Baliraine, Gregory D Frederick

Genome annotation of the temperate phage Phalm revealed two genes with homology to methylase/methyltransferase (MTases) genes in other bacteriophages, as well as in various soil-dwelling bacteria. MTases are enzymes that methylate specific bases in nucleic acids. In bacteria, MTases are known to be important to cell survival and other aspects of nucleic acid metabolism. MTases also function in epigenetic regulatory processes. Further, MTases function in restriction-modification systems. For proper cell function, nucleic acid modification is essential in directing the monitoring of the genome by other cellular enzymes. Although the functions of bacterial MTases are well known, the exact functions of MTases in bacteriophage genomes remain unelucidated.

In phage Phalm and other members of the P1 subcluster, such as Brusacoram and Shipwreck, MTases genes are located next to proteins of unknown function. Similarly, in *M. abscessus subsp. bolletii strain 107*, MTase genes are surrounded by hypothetical proteins. Notably, within two or three genes on either side of the MTase genes in Phalm and other subcluster P1 phages lies a gene encoding an endonuclease or a helix-turn-helix DNA binding domain protein. Moreover, one of the hypothetical proteins in *M. abscessus* is homologous to gene 51 of phage Phalm, and in this region synteny is very similar. It is hypothesized that MTases are most likely involved in protecting the phage genomes inside their bacterial hosts. Specifically, MTases most likely protect the phage genome from restriction by host enzymes. Previous investigations indicate that MTases are necessary for stable lysogeny. Characterization of phage-encoded MTases could have relevance in host-range determination. NCBI BLASTp analysis of Phalm gp 53 and 55 align with homologs in multiple soil-dwelling bacteria. These bacteria include *Mycobacterium sp. UM\_RHS*, *M. abscessus*, *M. salmoniphilum*, *M. chelonae*,

*M. fortuitum*, and *Rhodococcus*. Phalm gp 53 and 55 aligned with different site-specific MTase genes in *M. abscessus*. The presence of two MTases homologous to slightly different bacterial proteins implies that phage Phalm inhabited bacterial species possessing more than one restriction system. The acquisition and preservation of multiple MTase genes would protect subsequent generations.

MTases genes in clusters N, AY, O, and AQ were also examined. The MTase genes in these phages also showed homology to multiple bacterial MTases. These gene products showed homology to bacterial MTases, specifically those of the same genus as the host species used in isolation.

This study describes application of multiple bioinformatics tools, including Phamerator, NCBI BlastP, HHPred, and Splitstree, to elucidate plausible roles and significance of MTase genes in bacteriophage genomes.

# 11th Annual SEA Symposium Abstract

Merrimack College  
North Andover MA

Corresponding Faculty Member: Janine LeBlanc-Straceski (janine.leblancstraceski@merrimack.edu)



**Morgan Murray**

## ***You Are One in a Minion(Dave): A Comparison of F1 Cluster Bacteriophages***

**Morgan Murray**, Madison Carney, Annmarie Schmid, Charlotte Berkes, Janine LeBlanc-Straceski

MinionDave, Piper2020, and Cornucopia are three *Mycobacterium smegmatis* bacteriophages of the Siphoviridae morphology found in the F1 cluster. In Spring 2019, students at Merrimack College annotated the genome of MinionDave, which was isolated from Cathedral Caverns State park near the Mystic River in Alabama by a student at Jacksonville State University in 2013. Two other F1 cluster phage, Cornucopia and Piper2020, were isolated, characterized and annotated at Merrimack College. A comparative analysis of the genomes of MinionDave (58027 bp), Piper2020 (57703 bp) and Cornucopia (55422 bp) was performed. MinionDave is homologous with both Piper2020 and Cornucopia in the first half of the genome beginning at approximately 7000 bp, while Piper2020 and Cornucopia are homologous throughout this region. Divergence in genome structure at the 5' end is consistent with a previous analysis of the F1 cluster (Hatfull, et al. J Mol Biol. 2010 Mar 19; 397(1): 119–143.). Further investigation shows that the first 8 genes of MinionDave are found in a subset of bacteriophage in the F1 cluster such as CaptainTrips, MilleniumForce, and KristaRAM. MinionDave and Cornucopia have Y integrase genes belonging to Pham45734, whereas Piper2020 contains a Y integrase in Pham44984. These are the only two integrases present in the F1 cluster, although they are represented in a subset of other clusters as well (Pope et al. 2011. PLoS ONE 6(10): e26750. doi:10.1371/journal.pone.0026750.). We have observed that Piper2020 generates clear plaques and Cornucopia generates cloudy plaques consistent with lysogeny. Therefore we predict that MinionDave would also be a temperate phage based on the similarity of its integrase to Cornucopia. BLAST comparison showed MinionDave and Cornucopia's integrases were 91% similar. All three phage have different DNA methylase genes belonging to four different Phams. Piper2020 has two long DNA methylase genes (1518 bp and 2520 bp, respectively) but Cornucopia and MinionDave each have only one medium sized DNA methylase (951 bp and 1302 bp, respectively). In addition, MinionDave and Cornucopia have 105 genes, but while their genomes are roughly the same size they differ by about 3000 base pairs in length. Based on the comparisons of Cornucopia, Piper2020, and MinionDave a more in depth understanding can be reached regarding the function of F1 clusters and their relationship to *Mycobacterium smegmatis*.

# 11th Annual SEA Symposium Abstract

Miami University  
Oxford OH

Corresponding Faculty Member: Mitchell Balish (balishmf@miamioh.edu)



**Hope A Kirby**

## ***Two new *Microbacterium foliorum* bacteriophages, Belthelas (EE) and Lupine (ED1)***

**Hope A Kirby**, Natalie L Hanson, Leah R Watson, Hannah R Wilson, Meili A Aiello, Faith L DeVengencie, Rocco A Huston, Avery M Imes, Halie M Leftwich, Maeve E Proto-Newton, Brandon E Romell, Maria R Schlegel, Maddy D Spencer, Sydney Arlis, Garrett M Schilling, Elizabeth Lucas, Mariah S Squire, Kelly Z Abshire, Rebecca S Balish, Mitchell F Balish

In Miami University's first efforts at discovering phages that infect *Microbacterium foliorum*, 10 students out of 20 successfully isolated phages from samples of soil and leafy organisms. All 10 phages had morphology typical of Siphoviridae. Two lytic phages, Belthelas and Lupine, were submitted for genome sequencing and subjected to annotation. In agreement with predictions from DNA analysis, Belthelas was found to be a cluster EE phage with a typical small genome of 17,502 bp and 25 predicted genes. The gene content, more or less identical to most other cluster EE phages, confirms the low diversity within this phylogenetic cluster, which is well-represented in the Actinobacteriophage Database. Lupine, in contrast, was a member of the much less commonly described subcluster ED1, with a genome of 62,533 bp. Preliminary annotation suggests approximately 110 genes plus 7 present in a second copy on the 3,260-bp terminal repeat region of the linearized genome. Analysis of these genes suggests substantial diversity within subcluster ED1, with an assortment of genes not assigned to any pham and some others that, although assigned to phams, have no BLAST hits above the threshold of significance. None of these novel genes could be assigned likely functions. We conclude that whereas the phages of cluster EE, frequently encountered and bearing low diversity, have arrived at a highly successful strategy for propagation, the highly diverse phages of subcluster ED1 are rather dynamic in terms of evolution.

# 11th Annual SEA Symposium Abstract

Minnesota State University Moorhead

Moorhead MN

Corresponding Faculty Member: Sumali Pandey (sumali.pandey@mnstate.edu)



**Kelsey Leach**

## ***Isolation and Characterization of Microbacterium phage Etta and Discovery of Bacteriophage Vers Using a Novel Antarctic Cryobacterium Isolate***

Anna Madsen, **Kelsey Leach**, Madelyn Madsen, Sumali Pandey, Michelle Tigges

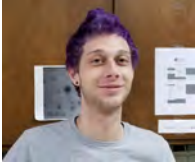
Bacteriophages are some of the most prolific and diverse beings on the planet, however, comparatively little is known about their diversity. The aim of this project was to understand the diversity and characteristics of phages that infect members of the Microbacteriaceae family of Actinobacteria. This family includes bacteria common to terrestrial plant and soil environments, such as *Microbacterium foliorum*, as well as psychrophilic bacteria common to glacial environments, such as *Cryobacterium*. Soil samples were collected from (46.866729 N, 96.75782 W), and direct isolation on *M. foliorum* was used to identify a cluster EA1 phage, Etta. Etta is a lytic phage from the EA cluster and the EA1 subcluster. It is a siphoviridae with small round plaques with low to moderate turbidity. Etta has a genome that is 41542 bp, with 63.3% GC content, and is closely related to phages Gelo (99% identity) and Calix (99% identity). To extend our analysis of Microbacteriaceae phage diversity, we developed methods allowing for the isolation of a phage through the infection of a novel Antarctic psychrophilic *Cryobacterium* isolated from a supraglacial stream. Water was collected from (44.872171 N, 91.938466 W) and traditional phage isolation methods were modified to create a protocol which would allow for the discovery of phages at 4°C, leading to the isolation of bacteriophage Vers. Interestingly, Vers is a phage with the ability to infect multiple bacteria in a cold environment and can be propagated at 4°C using both *Microbacterium foliorum* and *Cryobacterium* isolate as a host. This experiment provides insight into the diversity of phages and how characteristics and genetics vary in phages isolated from different environments and hosts.



# 11th Annual SEA Symposium Abstract

Mitchell Community College  
Statesville NC

Corresponding Faculty Member: Parks Collins (pcollins@mitchellcc.edu)



**Thomas Ray**

## ***LaviMo: Isolation, DNA Analysis, and Comparative Genomics***

Thomas Ray, Ryan Kistemaker, Alyssa Moliis

As part of the HHMI SEA-PHAGES program, over 200 Microbacteriophages have been identified, sequenced and classified into clusters. Mitchell Community College in Statesville, NC just completed its first year as part of the HHMI SEA-PHAGES program. In the fall semester, students isolated seven bacteriophages from soil samples. The phage LaviMo was chosen to be sequenced. LaviMo was isolated through direct isolation. Electron microscopy and molecular characterization indicated that LaviMo belonged to Siphoviridae. DNA from LaviMo was isolated and the complete genome was sequenced during the spring semester. Auto-annotation through DNA Master revealed twenty-six open reading frames. Based on average nucleotide identity, LaviMo is classified as a cluster EE microbacteriophage with a 99% sequence identity to phages Scamander and BurtonThePup. Here we present the initial data from the annotation of this new cluster EE phage. This research expands the diversity among cluster EE phages and provides an insight to the evolutionary characteristics of microbacteriophages.

# 11th Annual SEA Symposium Abstract

Monmouth College

Monmouth IL

Corresponding Faculty Member: James Godde (jgodde@monmouthcollege.edu)



**Sarah A Poirier**

## ***Isolation and Genomic Annotation of Novel Microbacterium Phages: Is the EC Cluster Losing Its Tail Assembly Chaperone Slippery Sequence?***

Brendan G Guenther, Ashley N Long, Piper N Miller, Jordan M Peckham, **Sarah A Poirier**, Eric M Engstrom, James S Godde, Timothy J Tibbetts

In 2018 Monmouth College joined SEA-PHAGES (Cohort 11). To contribute to expanding the number and diversity of well-characterized phage genomes, we are working to isolate novel *Microbacterium foliorum* bacteriophages. One phage we discovered, PiperSansNom, is a novel member of the EC cluster. In evaluating the tail assembly chaperone translational frameshift “slippery” sequence of PiperSansNom, we concluded that this phage lacked a slippery sequence. However, we noted that a minority of EC phages exhibit annotated slippery sequences, specifically CCCCCTA. In all other *Microbacterium* clusters, either all cluster members exhibit annotated slippery sequences, or all clusters lack these sequences. We performed phylogenetic analysis on the first (universally transcribed) EC tail assembly chaperone genes. Relationships of these genes suggest a loss of slippery sequences within the EC cluster.

# 11th Annual SEA Symposium Abstract

Montana Tech of the University of Montana  
Butte MT

Corresponding Faculty Member: Marisa Pedulla (mpedulla@mtech.edu)



Winter R Kempainen

## ***Investigation of 23 different Mycobacterium smegmatis lysogen strains***

Winter R Kempainen, Riley D Hellinger, Joel W Graff

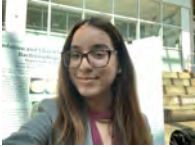
Twenty-three *Mycobacterium smegmatis* lysogen strains were created and grown using phages from 2018 SEAPHAGES laboratories. The bacterial cells and colonies of lysogenic and wildtype bacteria were visualized using Acid-Fast staining and colony growth on 7H10+++ agar plates. Each lysogen and the wildtype *M. smegmatis* were grown on 7H10+++ agar plates at 26°C, 30°C, 37°C, 42°C, and 50°C for 7 days. The plates were examined once per day. Lysogenic and wildtype *M. smegmatis* culture samples were treated with EtBr (2µg/mL) at 8 hrs., 16 hrs., and 24 hrs. of growth. The cells were visualized at 400x magnification with fluorescence microscopy. Three lysogen strains and wildtype were cultured in different 7H9 media of different CaCl<sub>2</sub> and glycerol concentrations. OD readings were taken over 3 days of culture growth. Among the lysogen strains, differences in colony morphologies and growth rates were observed.

# 11th Annual SEA Symposium Abstract

Montclair State University

Montclair NJ

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



Ashley S Peralta

## ***Isolation and Characterization of Novel *Arthrobacter* sp. and *Mycobacterium smegmatis* Bacteriophage from New Jersey Soil***

Ashley S Peralta

The goal of this study was to isolate and characterize novel bacteriophages from New Jersey soil samples that infect *Arthrobacter* sp. and *Mycobacterium smegmatis*. Soil samples were collected from diverse locations in northern New Jersey. Bacteriophage infecting *Arthrobacter* sp. and *M. smegmatis* were successfully isolated from enriched soil samples that were screened for their ability to form plaques on separate lawns of both species of bacteria. DNA was extracted from high titer lysates. Of the phages isolated in 2018, one *M. smegmatis* phage (Gator) and one *Arthrobacter* sp. phage (Linus) were sequenced and annotated in 2019. Gator is a cluster E temperate phage with a putative 133 genes (including two tRNA genes). Gator is the first temperate phage to be isolated at MSU as part of the SEA-PHAGES genomics course. Linus is a cluster AR lytic phage with a putative 109 genes. Like other AR phages, all but five of Linus's 109 genes are transcribed using the top strand of DNA, with the five genes transcribed from the bottom strand surrounded by forward genes.

# 11th Annual SEA Symposium Abstract

Mount Saint Mary College  
Newburgh NY

Corresponding Faculty Member: Suparna Bhalla (suparna.bhalla@msmc.edu)



**Eleanora G Robinson**

## ***The Journey Continues: Annotation of three novel Microbacteriophages Stanktossa, DirtyBubble and Roman***

Eleanora G Robinson, Suparna Bhalla, Evan Merkhofer

In 2017-2018 the Mount Saint Mary College Phage Hunters transitioned to the host *Microbacterium foliorum* for the isolation on novel bacteriophages in an attempt to provide more insight into the diversity of genomes across the Actinobacteriophage phylum. This year, fifteen Microbacteriophages were isolated from soil samples gathered in Newburgh, NY. Phages Stanktossa and DirtyBubble were obtained through enriched isolation, both yielding small, cloudy plaques after 48 hours of incubation at 30°C while Roman was obtained as a result of a direct isolation, yielding large, clear plaques after 24 hours at 30°C. TEM analysis revealed that all three phages have the Siphoviridae morphotype. Sequencing of Stanktossa (circularly permuted, 41.8 kB length, 63.6% GC content), DirtyBubble (linear with 3' sticky overhang, 41.6 kB length, 68.7% GC content) and Roman (linear with direct terminal repeat, 64.2 kB length, 62.8% GC content) identified them as members of the EA1, EB, and ED1 subclusters, respectively. All three phages were predicted to have a lytic life cycle based on the initial analysis of the genome sequences. Successful annotation of these novel phages was a collective effort between several students and faculty using bioinformatic programs including PECAAN, GeneMark, Phamerator and Starterator. Gene functions were assigned using the HHPred and NCBI BLAST programs. In addition, we are performing phylogenetic analysis of the three bacteriophages to determine their evolutionary relationship. We are also characterizing the phage Goldina, which currently exists as a mixed sample with Roman. Using genomic FASTA files from both Goldina and Roman, we are using phage-specific primers and PCR to make separate pure titers of the two phages. Upon successful separation, the Goldina genome will be annotated.

# 11th Annual SEA Symposium Abstract

Neumann University

Aston PA

Corresponding Faculty Member: Matthew Mastropaolo (mastropm@neumann.edu)



**Christopher J Negro**

## ***An Expedition in the Leaves, the Characterization and Annotation of Microbacterium Phage Hiddenleaf.***

**Christopher J Negro**, Daekwon M Sequira, Megan Bates, Christian Bjorkelo, Trang Doan, Hailey A Johnson, Nafees Norris, Emily Sasher, Ian M Sigmund-Hamre, Courtney L Womack, Christina T Zacconi, Lauren R Salvitti, Patricia Fallest-Strobl, Matthew D Mastropaolo

In the 2018-2019 academic year eleven students at Neumann University collected soil and water samples from Delaware County, PA. *Microbacterium foliorum* NRRL B-24224 SEA was used as the host to isolate 10 bacteriophage as part of the first cohort of SEA-PHAGES students. Hiddenleaf was isolated and purified from a soil sample at the base of a garden bird bath under some chive plants. The phage was separated using standard procedures to isolate a genetically unique phage sample and amplified. DNA extraction and sequencing were performed. Analysis of the genome classified Hiddenleaf as Siphoviridae morphology in the EF cluster, which currently has 7 total members, 6 of which are currently annotated. Hiddenleaf has 84 genes and a genome length of 56082 bp, which is the smallest genome of the EF cluster. Seven orphans were identified in the genome. The genome was annotated using PECAAN, NCBI BlastP, HHPred and a comparative analysis was done using phagesdb.org and phamerator.org.

# 11th Annual SEA Symposium Abstract

North Carolina A&T State University  
Greensboro NC

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



Anjali Kumari

## ***Bacteriophages Marteena and Nubi: Distinct Clusters United by Shared Phams***

Anjali Kumari, Ria Holloman, Olufemi Olatidoye, Rachel Richards, Jewel Washington, Correggio Peagler, Lia Artis, Lauren Thompson, Abeku Abercrombie, Lindsey Adams, Kevin Frazier, Najee' Green, Roy Coomans

Bacteriophages are viruses that replicate within a bacterial host. Due to their size and mode of replication, phages are far more abundant than bacteria or any other organism. We isolated six bacteriophages of *Gordonia terrae* CAG3 in the fall of 2018. DNA extracted from one of these, Marteena, was submitted to the Pittsburgh Bacteriophage Institute for sequencing. Marteena was isolated from soil collected on the campus of North Carolina A&T State University. Marteena is in subcluster CY1. Its genome is 50531 base pairs in length with a 66.6% GC content. Through isolation, purification, and computational analysis, we were able to articulate the similarities and differences between Marteena and Nubi, a second bacteriophage of *Gordonia terrae* isolated at N. C. A&T in the fall of 2017. Nubi, a cluster DC phage, has a genome length of 58718 base pairs and a 67.9% GC content. Nubi and Marteena exhibit a temperate life cycle. Some host cells are lysed following infection, releasing newly replicated phage particles, while other host cells become lysogens. Our research involved two major activities, isolation of the phage and annotation of the genome. The initial process of isolation was accomplished through enriched isolation and amplification of the phage particles using serial dilutions. DNA Master, GeneMark, HHPred, NCBI BLAST, phagesdb, Phamerator, I-TASSER, SEA-PHAGES.org, and PECAAN were employed to annotate, compare, and hypothesize on the function of genes and origin of differences present in the two phages. Phylogenetic trees generated from single gene comparisons allowed us to evaluate the placement of Nubi and Marteena into clusters and sub-clusters. BLAST revealed that Nubi and Marteena contain genes similar to those found in a variety of different bacteria. Could this be due to the evolutionary advantage of producing these bacterial proteins, or is it a result of phages assimilating their DNA into the bacterial DNA to hijack replication and produce more phage particles? Even though phages might have a negative reputation, they can also be beneficial through manipulation and utilized to cure lethal bacterial infections. The research, in collaboration with the SEA-PHAGES Program, allows undergraduate students to articulate information through analysis and hypothesize new information.

# 11th Annual SEA Symposium Abstract

Northwestern College

Orange City IA

Corresponding Faculty Member: Sara Tolsma (stolsma@nwcsiowa.edu)



**Kristina M Sevcik**

## ***Investigating Our Phage-Filled World: Discovery, Annotation, and Antibodies***

Kristina M Sevcik, Byron Noordewier, Sara S Tolsma

Six bacteriophages previously isolated at Northwestern College using *Mycobacterium smegmatis* mc2155 as host were sequenced: DrLupo, JacoRen57, Beelzebub, and RedRaider77 using RFLP-directed DOGEMS and Antonia and Raela individually. We annotated all six mycobacteriophage genomes. In addition, we isolated eight phages with *Microbacterium foliorum* as host and one phage with *Gordonia terrae* as host. These have yet to be sequenced. Our annotation efforts focused on the six discovered phages and four adopted phages: Chelms, Laila, Phineas and Cracklewink. All annotated phages are siphoviridae in morphology with only Phineas and Cracklewink being temperate. Collectively they represent eight different clusters. Antonia was typical of the abundant cluster B1 phages. DrLupo is related to Barnyard, the other cluster H2 phage. It has a non-canonical frameshift in its tail assembly chaperone gene, several orphans, and a low GC content of 57.5%. JacoRen57 is a singleton, most closely related to the AB cluster phages FF47 and Muddy. Its long tape measure gene is an orphan, there are numerous orphans with unknown functions in the right arm of the phage, and its GC content is low (56.7%). Since FF47 and Muddy can infect *Mycobacterium abscessus*, the Hatfull lab is working to see if JacoRen57 can also infect related hosts. Beelzebub, Raela, and RedRaider77 are cluster S phages. All three contain minor tail protein genes in their right arm as do other cluster S phages and previously confirmed in phage Marvin. Phineas resembled other P1 phages—especially Shipwreck and Fishburne. Cracklewink is more closely related to Bipper than Typha, the other two cluster Y members, especially in the right arm of the genome. It has one tRNA gene and some interesting large gaps that lack coding potential. Chelms is a cluster CS *Gordonia* phage. It is typical of cluster CS phages with a two-part lysin A gene (N-acetylmuramoyl-L-alanine amidase domain followed by the protease domain) and a lysin B 15 genes downstream of the two-part lysin A. It contains both forward and reverse genes and a single tRNA gene. Laila is cluster AN *Arthrobacter* phage. At 15,556 bp, it was the smallest of our phages. Laila exhibits synteny typical of cluster AN phages with a single tail assembly chaperone gene, adjacent lysin A genes (L-Ala-D-Glu peptidase domain followed by the N-acetylmuramoyl-L-alanine amidase domain), a single reverse gene encoding a helix-turn-helix DNA binding domain protein, and an HNH endonuclease at the far right arm of the phage. We



annotated an often-missed gene immediately upstream of Laila's tape measure gene. Finally, we continue working to characterize our anti-mycobacteriophage protein serum and perform fusion experiments to establish hybridomas that produce monoclonal antibodies against mycobacteriophage proteins.

# 11th Annual SEA Symposium Abstract

Nyack College

Nyack NY

Corresponding Faculty Member: Jackie Washington (jacqueline.washington@nyack.edu)



Angela M De Jesus

## ***Non-Mycobacterial Actinobacteriophages Providing More Insight to Phage Biology***

Angela M De Jesus, Mariana P Moraes, Jailyne E Aguilar, William S Cruz, Rebekah E Graham, Morgan A Hans, Hannah R Klumb, Julianna R Kranes, Joey F Leveroni, Thaina Petit-Frere, Nicolas J Plaja, Yonel A Rodriguez, Desiree A Torres, Zachary L Trador, Benjamin Wasem, Peter J Park, Jacqueline M Wasington

Isolation and characterization of non-mycobacterial actinobacteriophages continue to increase our knowledge about bacteriophage biology. This year at Nyack College we isolated a total of fifteen phages, which included eight *Gordonia terrae* phages, two *Rhodococcus* phages and five *Microbacterium* phages isolated using *Microbacterium paroxydans* and *Microbacterium foliorum* as hosts. One *Microbacterium* phage DizzyRudy (EL) and two *Gordonia* phages Ewald (DT) and MintFen (CV) were sequenced. Of these, DizzyRudy seemed to be particularly interesting as there are only 2 other members of the cluster, all having a minimum of 5 novel genes. DizzyRudy is 55, 815 bp with 89 genes, including several putative major tail genes and numerous predicted membrane proteins. The phage does not contain an immunity repressor or gene such as an integrase which would allow it to be maintained in the host cell as a prophage. No lysogens have been recovered.

Most bacteriophages have a narrow host range and typically only infect one bacterial host but some phages have a wider host range. Consequently, host range experiments were performed using several *Microbacterium* strains including *M. chocolatum*, *M. testaceum*, *M. radiodurans*, *M. saperdae*, *M. ketosidreducens* and *M. arborescens* to determine if any of our isolated bacteriophages were able to cross infect other species. MaeLinda which was isolated using *M. foliorum* was able to infect *M. ketosidreducens* with an efficiency of plating of 1. To understand this we performed phylogenetic analysis of these strains based on 16S rRNA sequences to determine the degree of relatedness to *M. foliorum* and the results will be presented.

To date, the nature of most receptors used for actinobacterial phage infections are not known and in addition, we do not understand why some bacteriophages have a wider host range. Therefore, understanding this will give us insight into bacteriophage – host interactions. The first step of bacteriophage infection of a host is recognition of a specific receptor which may be protein, carbohydrate or other cell membrane associated structures. To determine the type of receptors used by different bacteriophages, the results of ongoing

experiments will be presented. Preliminary experiments have shown that the *Gordonia* phage Ewald(DT) has a reduced ability to infect the host bacteria in the presence of spermidine. Polyamines such as spermine and spermidine have been shown to block porins present in bacterial cell wall used by some phages as a port of entry into the cell. This result suggests that a porin is required for Ewald to infect *Gordonia terrae*. We will also present our results of experiments to identify specific bacterial receptors using transposon mutagenesis.

# 11th Annual SEA Symposium Abstract

The Ohio State University

Columbus OH

Corresponding Faculty Member: Sarah Ball (ball.1766@osu.edu)



**Sabré Randall**

## ***Singleton No More: MargaretKali Finds a Match in Kumotta***

Sabré Randall, Sarah Ball, Caroline Breitenberger, Charles Daniels

During our eight years of participation in the SEA-PHAGES program, students from The Ohio State University have isolated over 1,000 bacteriophages, with 171 being archived and entered on Phagesdb.org and 48 sequenced genomes representing 12 clusters. In 2017, we isolated our first singleton, MargaretKali, using the host *Arthrobacter* sp. 21022, but it did not remain a singleton for long. Kumotta, which was isolated from a soil sample collected in 2018, was found to have enough similarity to MargaretKali to form a new cluster, FB. Kumotta's genome is 40,3017bp in length with a GC content of 60.8% (compared to MaragretKali's 39,448 bps and 61.1% GC content). The two genomes share a nucleotide similarity of 98% over the majority of their lengths, except for the first nine open reading frames (~7800bp), a span of about 2,300bp in the middle of the genomes and the final 1,000bp. Kumotta contains 70 predicted open reading frames, of which 15 are still classified as orphans despite the similarity to MargaretKali. The majority of these orphans have BLASTP hits to hypothetical proteins of several Actinobacteria genera, perhaps suggesting the presence of prophages.

# 11th Annual SEA Symposium Abstract

Ouachita Baptist University

Arkadelphia AR

Corresponding Faculty Member: Ruth Plymale (plymaler@obu.edu)



**Thomas R Harrington**



**Noah R Thompson**

## ***Lysogenic host bacterium alters plating efficiency of *Gordonia* bacteriophage***

**Thomas R Harrington, Noah R Thompson,** Hayden H Bowman, Gracie C Jones, Elijah J Begin, Erin E Chappell, Gracen L Hambrick, Allie E Harris, Bennett L Hasley, Cade M Haynie, Griffin A Hopkins, Carlee B Hutchins, Dane A Jester, Jenny J Johnson, Allison P Martin, Kevin D Merino, Celeste N Pinkerton, Gabe Poe, Taylor D Savage, Zane Smith, Hunter Smith, Timothy A Spiva, Libby L Thompson, Ryane E Thurman, Caleb T West, Nathan S Reyna, Ruth C Plymale

Bacteriophage infecting *Gordonia terrae* 3612 or *Gordonia rubripertincta* NRRL B-16540 were isolated from soil samples by Ouachita Baptist University students and the *Gordonia* host range of each phage was determined. Most phage were able to infect both *Gordonia* species but displayed a higher plating efficiency on the isolation host. During the host range screening process, we observed lysogen formation by *G. terrae* bacteriophage DelRio and Ruthy on both *G. terrae* and *G. rubripertincta*. We harvested virions from all four lysogens—*G. terrae* (DelRio), *G. rubripertincta* (DelRio), *G. terrae* (Ruthy), and *G. rubripertincta* (Ruthy)—and plated them on both *Gordonia* species. Lysogen bacterial species had a marked influence on infectivity, with virions isolated from *G. terrae* lysogens exhibiting a drastic reduction in plating efficiency on *G. rubripertincta*, whereas virions derived from *G. rubripertincta* lysogens infected both *G. terrae* and *G. rubripertincta* with similar plating efficiency. This differential infectivity was observed immediately after lysogen creation, suggesting host-induced impacts to phage protein expression or post-transcriptional modification rather than changes to phage genome sequence. Virions harvested from each lysogen are being analyzed using mass spectrometry and results of that analysis will be presented.

# 11th Annual SEA Symposium Abstract

Providence College

Providence RI

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**Colby Agostino**

## ***Isolation of 15 novel mycobacteriophage from soil on the campus of Providence College***

Colby Agostino, Ethan Dionne, Olivia Schmitt, Samantha Oltavaro, Maxwell Sinoway, Kathleen Cornely

Our research team isolated 15 novel mycobacteriophage from soil collected on the campus of Providence College. *Mycobacterium smegmatis* was the host bacteria utilized during experimentation. Infecting *M. smegmatis* with phage was necessary to proliferate the population of phage and purify the phage populations. Multiple rounds of purification were necessary to obtain a homogeneous population of each novel phage. The plaque morphologies of each novel phage, which were determined after purification, were used as evidence to determine whether the phage reproduction cycle was lytic or temperate. The DNA from each phage was extracted and a restriction digest of the phage DNA was then performed using a HaeIII restriction enzyme; the restriction digest was necessary to ensure that each phage was indeed a novel phage. The DNA from each phage was also analyzed via PCR. The detection of a PCR product was used to determine if any of the phage belonged to clusters B, C, or F. Observation of the phage via electron microscopy confirmed that the morphotype of all of the phages was Siphoviridae. Two of the phages, Zolita and Skippy, were selected to have their genomes sequenced at the University of Pittsburgh. Our research team then annotated the genomic sequences of Zolita and Skippy to determine the location and function of genes found in their genomes. The data collected in this experiment advances the search for a method to treat *Mycobacterium tuberculosis*.

# 11th Annual SEA Symposium Abstract

Purdue University

West Lafayette IN

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



Lauren Novak

## ***Comparative genomic analysis of mycobacteriophages Krili, Corazon, Kanye, Nitzel, and Smooch***

**Lauren Novak**, Avantika Ajey Bhardwaj, Serena Birdinc, Katelyn Bormett, Morgan Callin, Misbah Chagpar, Elijah Cline, Ivy Crank, Rachel Damage, Julianne Dejoie, Davide Delisi, Nathan Everett, Chao Fu, Shruthi Garimella, Anna-Nikol Georgiev, Mikaela Hand, Alexander Harris, Ben Howard, Jenna Ischinger, Matthew Jaeger, Kierra Jammer, Jin Jang, Cindy Jiang, Liam Johnson, Connor Jones, Gwen Joseph, Eung Baek Kim, Emma Lietzke, Mai Liu, Sarah Liu, Estefania Martinez, Melanie Martinez, Brittney Mavrenovic, Korie McCrea, Marina Mehling, Alex Murfee, Abigail Murphy, Kathryn Myers, Quynh Nguyen, Maddie ONeill, Lauren Oparah, Paula Pandolfi, Nikita Patil, McKenna Pineau, Julio San Martin Lopez, Emily Sanders, Gabby Selvia, Kylie Snyder, Taylor Sorrell, Sharonluz Torres, Lina Trigg, Kiersten Troyer, Kess Turner, Bach Vu, Remington Wilson, Ryan Wollensak, YinTae Wyss, Eunhui Yoo, Neil Zhao, Sarah Bell, Janice Ying Li Chan, Emily Kerstiens, Joseph Krampen, Austin Larson, Mikael Reuhs, Jacob Riedel, Christina Sanchez, Gillian Smith, Ikenna Okekeogbu, Kari L Clase

Mycobacteriophages (hereafter referred to as phages) are ubiquitous viruses that infect mycobacteria. They have potential uses in the field of biotechnology and medical science with applications ranging from disease diagnosis, through phage typing, phage vaccine and phage therapy. Meanwhile, only a meager number of phages have been identified and characterized out of the multitudes present in the biosphere. In addition, a far majority of the bacteriophage genes that are discovered have no known function. In this study, five novel phages namely, Krili, Corazon, Kanye, Nitzel, and Smooch were annotated per the most recent guidelines using both PECAAN and DNA Master. While Krili was isolated and characterized at Purdue University, the other four phages were adopted from other institutions (see PhagesDB.org) for annotation. Of the characterized phages, Nitzel, classified as a cluster F phage, has the smallest genome size of 54kbp, and GC content of 61.3%. Corazon, a cluster S phage with genome size 65kbp has a GC content of 63.4%; Krili and Smooch from cluster O, both have a genome size of 71kbp and a GC content of 65.4%; while Kanye, a cluster E phage has a genome size of 75kbp and GC content of 63.1%. All five phages have the Siphoviridae morphotype. Only Kanye has a temperate life cycle, while the other four have the lytic life cycle. The right arm of Nitzel is characterized by many small genes, most with an overlap of 4 bp. Kanye has two tRNAs, while Nitzel has only one tRNA. Corazon, Krili, and Smooch have no tRNA. The cluster O phages, Krili and Smooch have their genomes flanked by reverse genes on the 5' and 3' ends. Corazon has two holin genes with each located downstream of each lysin gene, while Krili, Nitzel, Kanye and Smooch have only one holin gene. Structural genes such as terminase,

portal, capsid maturation protease, scaffolding, major capsid, head-to-tail adaptor, major tail, tail assembly chaperone, and tape measure proteins were located in the 5' upstream region of the genomes of Nitzel and Kanye. However, for the genome of Corazon, Krili and Smooch, these structural proteins are located in the middle of the genome. Corazon also has an unusual gene organization with some of its minor tail proteins located in the far right arm of the genome. The genomes of Smooch, Krili, Nitzel, Corazon and Kanye have 88 (70%), 88 (68%), 55 (56%), 75 (67%) and 82 (58%) genes with no known function respectively. Nitzel and Kanye have more members in their phage clusters and a lower frequency of genes with unknown function. The clusters of Smooch, Krili and Corazon have fewer members which may explain the higher frequency of genes with no known function in comparison to Nitzel and Kanye.



# 11th Annual SEA Symposium Abstract

Queens University of Charlotte  
Charlotte NC

Corresponding Faculty Member: Jennifer Easterwood (easterwj@queens.edu)



**Irene Kuriakose**

## ***The Isolation and Annotation of Bacteriophages “MCubed” and “Nucci”***

Irene Kuriakose, Joanna Katsanos, Jennifer Easterwood

Bacteriophages “Nucci” and “MCubed” were discovered during Queens’ third year in the SEA-PHAGES program. Both phages were identified from soil samples collected from a chicken coop in Charlotte, NC. Using the host *Microbacterium foliorum* (*M. foliorum*), enriched isolation methods were carried out by students in the biology department to locate the presence of phage. Purification and amplification techniques followed. Plaques from “Nucci” were clear, round, and typically less than 1 mm while “MCubed” displayed round, hazy plaques that were approximately 1mm. Transmission Electron Microscopy revealed that both phages exhibit the siphoviridae morphotype. While “Nucci” has a head diameter of 60 nm and a tail of 121 nm, “MCubed” has a head diameter of 51 nm and a tail measuring 140 nm. After DNA extraction, the phages were sent to the Pittsburg Bacteriophage Institute for sequencing. Using the bioinformatics software DNA Master and resources HHpred, Starterator, Phamerator, and NCBI blastp, students annotated the genomes of “Nucci” and “MCubed”. Both phages belong to the EA cluster, with “MCubed” being one of six phages in the EA2 subcluster and “Nucci” being the only identified phage in subcluster EA10. “Nucci” contained 40,281 base pairs and a 63.7% Guanine-Cytosine (GC) content while “MCubed” contained 40,381 base pairs and a 62% GC content. Both genomes had 63 open-reading frames, with over half identified as reverse genes.

# 11th Annual SEA Symposium Abstract

Queensborough Community College  
Bayside NY

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



**Valesca Polycarpe**

## ***Annotations and Analysis of LilMoolah***

**Valesca Polycarpe**, Deidre Seepersaud, Muhabbat Ahmedova, Calli Atamian, Biling Chen, Yiching Chen, Winnie Chow, Samentha Duffault, Brettania Gordon, Kamaljeet Kaur, Prabhjot Kaur, Dhanmattie Khan, Amirabbas Maghsoudi, Nishat Neela, Jennifer Ogbozor, Anastasia Parks, Megan Pirtle, Yusra Raza, Pamela Joy Tabaquin, Divya Thakur, Roxine Tyndale, Britney Vasconcellos, Vincent Zhong, Urszula Golebiewska

Students at Queensborough Community College annotated and analyzed the genome of Mycobacterium phage LilMoolah, a phage discovered in 2017 by Chanel Turner, a student of Queens University of Charlotte in Charlotte, North Carolina. It was isolated from Mycobacterium smegmatis mc<sup>2</sup> 155. LilMoolah is a member of the Siphoviridae family, characterized by double-stranded DNA and a long, noncontractile tail. It belongs to cluster F, subcluster F1. Its genome is 58,136 base pairs in length and made up of 109 genes. Various bioinformatics programs were used for gene annotation and analysis, such as DNA Master, BLAST, Starterator, HHpred, Phamerator, and others. These were used to determine each gene's length, coding potential, reading frame, possible function, and possible relatives. The first half of LilMoolah's genome contains larger genes, many of which have well-defined functions; these include terminase, lysin A and lysin B, holin, major and minor tail proteins, tape measure protein, integrase, and others. However, 65 of LilMoolah's genes, many of which are found in the second half of the genome, are much smaller in size and have no defined function. Most of the genes are read in the forward direction. The closest relatives to LilMoolah are RitaG, Mattes, and Nivrat, with Nivrat sharing the highest number of homologous genes with LilMoolah.

# 11th Annual SEA Symposium Abstract

Radford University

Radford VA

Corresponding Faculty Member: Joy Caughron (jcaughron2@radford.edu)



**Connor Fox**

## ***Exploring the effects of environmental factors on the phylogeny of bacteriophages***

Maxwell Barney, **Connor Fox**, Pravin Garskof, Jonathan Gibson-Cromer, Maria Gonzalez, April Hiett, Kelly Hodges, Laneigh Jones, Clint Krysa, Autumn Roberts, Jessica Wyllie, Joyce Caughron, Robert Sheehy

The purpose of this experiment is to collect environmental data gathered during the initial collection of the chosen EA1 sub-cluster phages and use this data to find trend in environmental significance of phage survival and phage distribution. By taking the data found and documented during phage collections, the environmental trend of selected phages can be examined as well as the likely displacement patterns of phages. The distribution is then overlaid with the phylogenetic analysis of the phage genomes at specified open reading frames that contain tail protein structure products. Two regions were specified to be tested, one containing high similarity in sequence analysis and synteny and another containing a higher variability region with open reading frames with tail protein products in the chosen phages. Two additional open reading frames were of particular interest due to one being annotated as a large gap of over one hundred base pairs consistently in the EA1 cluster phages as well as another open reading frame that has a possibility of being a recent horizontal gene transfer due to the coding potential of the host and phage not lining up in an expected pattern. These analyses are done using information gathered on Phages DB, NCBI Blast, Phamerator, MEGA 7, and information supplied by the institutions where phages were collected. If environmental factors impact the distribution and survival capability of phages, then the distribution of sub-cluster EA1 phages will likely overlap with phylogenetic analysis data of the following bacteriophages: Knox, Aubergine, HanSolo\_Draft, BonesMcCoy, Draft, Tenda, AlexAlder, Gello, Peep, Bonino, StingRay, Schubert, Gargoyle, and Dave.

# 11th Annual SEA Symposium Abstract

Rockland Community College  
Suffern NY

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



**Hannah Elizabeth**

## ***Digging for Diesel: A functional analysis of cluster A3 phage Dieselweasel***

**Hannah Elizabeth**, Nicole Esposito, Niola Etienne, George Joseph, Alexis Kangoonui, Michael Lenyk, Riley Luczkiewicz, Sherlin Mathew, Joseph Patyi, Sarah Pollak, Jeanine Siegel, Karen Umana, Ra'Vynn Waters, Jeff McLean

DieselWeasel is a lysogenic A3 phage with 87 genes, including 4 TRNAs. The evidence collected from bioinformatics tools such as PECAAN, Phaster and BLAST have shown that Dieselweasel can potentially integrate into mycobacterial species other than *M. smegmatis*. By ensuring adhesion to host receptors during the initial phases of infection, Minor Tail proteins are essential in the determination of host range in these bacteriophages. Mycobacteriophage Microwolf, a related A3 mycobacteriophage, is known for possessing a broad host range, but subcluster identification is not sufficient to identify host range. Therefore, we utilized Blastp to determine whether DieselWeasel (A3) or Chupacabra (A10) have a minor tail protein sequence similar to Microwolf. The alignment showed that Dieselweasel and Chupacabra have the same structure in the Gp5 protein sequence as Microwolf, indicating that these phages may also possess broad host-range. Further, it was discovered that Dieselweasel may have the potential to integrate into other medically important mycobacterial species other than *M. smegmatis*. Analyzing the genome of *M. tuberculosis* and *M. abscessus* (a multi-drug resistant and nontuberculous mycobacteria), it was found that the ATTB site of *M. abscessus* has a 98-100% similarity to that of the ATTP site of Dieselweasel, indicating the potential for Dieselweasel to form prophages in *M. tuberculosis* and *M. abscessus*. Disimilarity between the *M. abscessus*-derived prophages and Dieselweasel indicate that Dieselweasel may integrate into these bacterial genomes even in the face of superinfection. As a lysogenic phage, Dieselweasel and related phages use a stoperator system to inhibit lytic replication during lysogeny. Stoperators sole purpose throughout these sites are prevent lytic-associated gene transcription. Dieselweasel was discovered to possess the same Stoperator sequence as other A3 cluster phages (GTTCTCTGTCAAG). 9 Stoperators were located in Dieselweasel throughout the 87 genes with the associated Immunity Repressor found at gene #77. Similarly, A2 Mycobacterium phage Phaded also uses a stoperator system using the sequence (GGTGGATGTCAAG), similar to other A2 cluster phages. Six different stoperator sites and an immunity repressor were found in Phaded, of the 91 genes present in the phage. Additionally, as Dieselweasel is a lysogenic phage, it has fewer TRNA than most lytic phages such as HyRo(C1) and Alice(C1). Collectively the evidence suggests that Dieselweasel has potential for use in the detection and molecular manipulation of a broad range of mycobacterial species.

# 11th Annual SEA Symposium Abstract

Saint Joseph's University

Philadelphia PA

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



**Leya C Givvines**



**Paige V Conrad**

## ***There and back again with four Mycobacterium smegmatis bacteriophages.***

**Leya C Givvines, Paige V Conrad, Alexandra L Vlahovic, Arianna M Varano, Mia S Somershoe, April E Pivonka, Isabella Patterne, Shane M Patil, Teagan R McCarthy, Mary C Marino, Alec J Manzi, William R Majewski, Dana L Glavin, Tate J Fryczynski, Mary Agnus J Dunn, Vanessa D Copes, Joshua R Blackley, Elizabeth C Binder, Corinne O Merlino, Emily E Costello, Julia Y Lee-Soety**

The 2018-2019 cohort of Phage Safari students isolated and characterized 16 different mycobacteriophages that infect *Mycobacterium smegmatis*, a close relative of the tuberculosis-causing bacterium *Mycobacterium tuberculosis*. By restriction endonuclease digest patterns and transmission electron microscopy, nine phages exhibited short tails which classified them as myoviridae and likely belonging to the C1 cluster. The other 7 phages had long flexible, non-contractile tails which classified them in the siphoviridae family. Four phages were sequenced – Kipper29, Mcshane, Giuseppe, and JPickles. Kipper29 was sorted in cluster A6; its genome of 52,009 base pairs contains 98 genes with 3 genes encoding tRNA. Mcshane was sorted in cluster B1 with 68,929 base pairs containing 103 genes, including an orpham. Giuseppe was sorted in cluster D1 with 64,604 base pairs containing 89 genes. JPickles, with the myoviridae morphotype, belonged to the C1 cluster with 155,116 base pairs containing 228 genes, 36 tRNA genes, and one gene encoding tmRNA. Using both DNA Master and PECAAN, we have made gene calls and confirmed many proteins that they encode. So far, all four mycobacteriophages have very similar features as other phages in their respective clusters. After 4 years of attempting to isolate phages from other hosts – *Arthrobacter* and *Gordonia* – with low rates of success,

moving back to *M. smegmatis* gave every student in the class opportunities to carry out the entire phage isolation process and gain a sense of accomplishment.

# 11th Annual SEA Symposium Abstract

Saint Leo University

Saint Leo FL

Corresponding Faculty Member: Iain Duffy (iain.duffy@saintleo.edu)



Hailey R Kerns

## ***SEA-PHAGES at Saint Leo University: A Roaring Success! Discovery of Novel Phage, Comparative Analysis and Evaluation of EE and EK Cluster Bacteriophage Sequences.***

Hailey R Kerns, Carissa R Brown, Andrea Gemmati, Laela A Ouellette, Kaishon M Showers, Lisa Ogueke, Emily Katz, Victoria Latalladi, John Duncan, Iain Duffy

In Fall 2018, Saint Leo University was inducted into Cohort 11 of the SEA-PHAGES Program. In our first year, a total of fourteen bacteriophage were isolated from soil samples, using the bacterium *Microbacterium foliorum* as host. The soil samples were acquired at various locations, from Florida to Maryland, with the home campus of Saint Leo University providing the most popular site. The isolation, purification, titer determination, and further assessments of the bacteriophage were done using protocols provided by the SEA-PHAGE program. Plaque morphologies were ascertained, and transmission electron microscopy performed. RFLP analysis of bacteriophage genomic DNA was also undertaken, in addition to the utilization of a host specificity assay. Sequencing data from three of those phage were obtained and analyzed. One was an EE cluster phage [Kaijohn]. This cluster currently has 28 members, of which, 14 have been annotated. Kaijohn is analogous in sequence and gene arrangement to its closest cluster phamily members, as seen in Phamerator. Kaijohn would also appear to share the gene 10 and 11 gene slippage motif for the formation of the tail assembly chaperone protein. The sequences of two further phage, both EK, were obtained. One of those genomes [Wesak] has been identified as an EK1 cluster member. At the time of obtaining the sequences only one previous EK1 phage, ArMaWen, had been annotated, however, there are three other draft EK1 genomes awaiting final annotation. It would appear that Wesak has a close relative in another draft phage, Tiny Timothy, however it would seem that both have several genes that differ from the annotated ArMaWen phage. While Wesak shares many similarities with Tiny Timothy, there are intriguing differences. These include the fact that both Wesak and Tiny Timothy have orphans at genes 16 and 17, however, both of those genes have initially been designated as distinct orphans. Gene 35 in Wesak has a similar relative in Tiny Timothy but is also seen in the phage Araxxi, an EM cluster phage, but these are the only known instances of those genes. While gene 36 in Wesak is again, a member of the same phamily as gene 36 in Tiny Timothy [the only two members of this phamily], gene 37 is seen in both but also in Burro, another EM phage. Wesak would then also seem to have

another orpham in gene 39. The other sequenced EK phage, Blue Rugrat, has not been recognized officially yet, and so has yet to appear in sites such as Phamerator. However, we have been able to use programs such as DNA Master and NCBI Blast to analyze the similarities and differences with Wesak.



# 11th Annual SEA Symposium Abstract

Salem State University

Salem MA

Corresponding Faculty Member: Amy Sprenkle (asprenkle@salemstate.edu)



Cassandra R Kysilovsky

## ***A Study in Contrasts; Annotation of an AR and J Cluster Actinobacteriophage from the Genome Exchange.***

Cassandra R Kysilovsky, Amy B Sprenkle

In the inaugural SEA-PHAGES Bioinformatics semester at Salem State University, we annotated two phage genomes from the Genome Exchange at the Actinobacteriophage Database. The phage genomes were selected to provide the optimal training in developing the curriculum around using the bioinformatics tools provided by the program. The first phage annotated was the *Arthrobacter* phage Mordred. Mordred is a lytic Siphoviridae found in Northampton, MA from a direct isolation. Mordred's genome is just over 70 kilobase (kb) pairs and is in the AR cluster. We found the genome to be very closely related to members of the cluster, with no orphans or unusual annotation challenges. The second phage was the *Mycobacterium* phage Hannaconda, a lysogenic Siphoviridae found in a potted succulent from a Pittsburgh, PA Ikea also from a direct isolation. Hannaconda is in cluster J, and is over 111 kb. This large genome was significantly less related to members in cluster J, which is characterized by large genomes with orphans, mobile genetic elements, intron splicing in capsid proteins, and tRNAs, all of which provide a much more significant genome annotation challenge. The online bioinformatics guide, DNA Master, Phamerator and PECAAN were all thoroughly explored and utilized to complete the annotation of our phage genomes.

# 11th Annual SEA Symposium Abstract

Southern Connecticut State University  
New Haven CT

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



**Stephanie Preising**



**Amelia Hoyt**

## ***Characterization of a new *Microbacterium foliorum* Cluster EB phage 'Stromboli': A tale of toxins, giant lysins, HNH endonucleases, and a gene cluster predicted to regulate nucleotide levels.***

**Stephanie Preising**, Zachary Williams, Karina Martinez, Lindsay Kashuba, Perpetual Taylor, Mia Forgione, Faruk Senturk, Eleanor Tinsley, Richard Szeligowski, Johnesha Brown, Shannon Barrett, **Amelia Hoyt**, Nicholas P Edgington

Stromboli is a new member of the Cluster EB, a cluster that infects the bacterial host *Microbacterium foliorum*. It has a 41,594 bp genome and a GC% of 68.8. It is one of eighteen members in this cluster, is in the *Siphoviridae* family, produces a 'bullseye' plaque morphology, and is predicted to be a lytic phage. It has an unusually large lysinA which contains several repeats. We will present a phylogenetic analysis of the Type II HicAB system predicted in *Microbacterium* and actinobacteriophages. Stromboli also contains two predicted HNH endonucleases, tRNAs, and several genes that are predicted to encode enzymes that regulate nucleotide levels in the bacterial host.

# 11th Annual SEA Symposium Abstract

Southern Maine Community College  
South Portland ME

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



**Matthew Davis**

## ***Seasonal lysogeny in temperate water marine phage***

**Matthew Davis**, Theodore Bishop, Lily Cox, Crystal Turner

Many temperate water marine phage are thought to form lysogens over the winter months. While searching for a marine Actinomeycete host in the Spring 2019 semester the team at Southern Maine Community College (SMCC) encountered difficulties infecting potential hosts isolated from the marine environment. Potential hosts were isolated from biofilms on marine invertebrates, macroalgae, rocks and sediments. The hosts were used in enrichment cultures in an effort to find phage specific to them. Hosts were gram stained and gram positive hosts were identified to genus by DNA barcoding a portion of the 16srRNA gene. All the gram positive hosts were in the Firmicutes and none of the enrichments yielded phage. We hypothesized that the majority of marine phage form lysogens in the cold months and designed an experiment to test this hypothesis. Three enrichments were done using biofilms from lobsters, tunicates and sediments obtained from the marine environment (ambient temperature 3-7 degrees C) and comparable sources from a heated, recirculating wet lab (18 degrees C). In each case no infection was observed from the 3 degree water and infection was observed in hosts isolated from biofilms in the 18 degree water thus supporting our hypothesis of cold temperatures leading to lysogeny. These results are discussed in the context of the “piggyback the winner” hypothesis of marine lysogeny.

# 11th Annual SEA Symposium Abstract

SUNY Old Westbury

Old Westbury NY

Corresponding Faculty Member: Christos Noutsos (noutsosc@oldwestbury.edu)

## ***mechanisms of Genome Expansion in Phages***

Iman Raja, Fernando Nieto, Christos Noutsos

Bacteriophages are viral organisms that propagate through the infection of bacterial hosts. Their ability to do so is influenced by the amount of DNA within their capsid. This indicates the presence of evolutionary pressures, leading towards gene-creating mutations. In this project, the mechanisms of genome expansion are studied that could potentially lead to new phage strains. Three modes of genome expansion were noted: Expansion of existing genes by inteins, Insertions of newly encoded genes, and *de novo* genes. For further investigation, models were developed. For the first mode, certain phams unique to each Cluster were repeatedly expanding among aligned pairs. After being tested for Synonymous/Non-synonymous substitutions, seven showed neutral/negative selection while two demonstrated neutral/positive selection. For the second mode, Insertions, the developed hypothesis indicates that the observed new genes were part of an ancestral phage that may have been passed down through positive selection. For the third mode, *de novo* genes were noticed throughout the phage genomes due to a few nucleotide deletions in their sequences. When those deletions were filled in, the genes disappeared. Overall, several factors causing genome expansion among Bacteriophages were revealed.

# 11th Annual SEA Symposium Abstract

Tarleton State University

Stephensville TX

Corresponding Faculty Member: Dustin Edwards (dcedwards@tarleton.edu)



**Faith Cox**

## ***Isolation of Cluster EA2 Bacteriophage Finny***

**Faith Cox**, Stacy Luka, Tiffany Lee, Michaela Aguirre, Shey Andrews, Kayla Bahr, Abigail Ballard, Matthew Bristerpostma, Leah Dowell, David Kiker, Tiffany Lujan, Haze Murphy, Abbigal Ramirez, Rheaven Sandoval, Kenneth Underhill, Julie Edwards, Dustin Edwards

Bacteriophage Finny was purified from *Microbacterium foliorum* at 29° C in PYCa media by direct isolation method from a soil sample collected from a chicken coop in New Braunfels, Texas. Following initial isolation, two rounds of serial dilutions and plaque assays were performed for bacteriophage isolation and purification. Bacteriophage Finny plaque morphology consists of small-to-medium-sized lytic plaques with turbid halo rings. High titer lysate was stained with uranyl acetate to visualize bacteriophage Finny by transmission electron microscopy, which showed the virus has Siphoviridae morphology with an icosahedral capsid. DNA was extracted by a zinc chloride method and the whole genome sequenced at the Pittsburgh Bacteriophage Institute. Whole genome sequence comparison determined that bacteriophage Finny is a Cluster EA2 cluster with a circularly permuted genome 40,313 bp in length with 62.1% G+C content. Bacteriophage Finny genome contains 63 predicted protein-coding genes, including lysin A, holin, RecA-like DNA recombinase, AAA-ATPase, MazG-like nucleotide pyrophosphohydrolase, thymidylate kinase, and ThyX thymidylate synthase.

# 11th Annual SEA Symposium Abstract

Truckee Meadows Community College  
Reno NV

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



**Abigail T Peterson**

## ***Characterization and Genomic Analysis of Mycobacteriophages Lewan and Heathen, Including Host Range Investigation of Phage Heathen***

**Abigail T Peterson**, Keziah Dutt, Heather Bohn, Kristen Sommerfeld, Ethan Pierrot, Deanna Lewis, Frank H Robertson, Tina Slowan-Pomeroy, Laura A Briggs

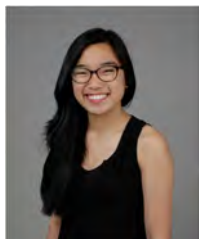
The goal of this research was to isolate, purify, and characterize bacteriophages found in Northern Nevada soils. In conjunction with the Howard Hughes Medical Institute's Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program, this research expands our understanding of the diversity of bacteriophages in this region. The focus of this study was on mycobacteriophages Heathen and Lewan. Both phages were isolated from soil in Reno, NV using the host *Mycobacterium smegmatis* mc<sup>2</sup>155. Heathen was found in 2015 and Lewan was found in 2018. Each phage was isolated and purified by the plaque purification method until uniform plaques were obtained. Lewan has turbid plaques ranging in size from 1-2 mm in diameter, indicating possible lysogeny. Heathen has bullseye plaques 3mm in diameter, also indicating possible lysogeny. Phage DNA was extracted from both lysates and sent for sequencing at the Pittsburg Bacteriophage Institute, followed by annotation using PECAAN and Phamerator. Lewan is a subcluster L2 mycobacteriophage with a GC content of 59.0% and a genome length of 76734 base pairs with 137 genes and 13 tRNAs. Heathen is a subcluster A3 mycobacteriophage with a GC content of 64.0% and a genome length of 50143 base pairs with 87 genes and one tRNA. Heathen contains 2 orphams and Lewan contains 4 orphams. Heathen shows 99.7% and 98.14% sequence similarity to A3 phages HelDan and Fred 313, respectively. Lewan shows 98.08% and 98.07% sequence similarity to L2 phages mkalimitinis3 and Crossroads, respectively. Host range analysis of 7 actinobacterial strains, showed Heathen can cross infect *Gordonia terrae* 3612, *Rhodococcus erythropolis* RIA643, *Mycobacterium phlei* NCTC8151 and *Mycobacterium tuberculosis* H37Ra. Host range testing for Lewan is pending. Heathen contains both a tyrosine integrase gene (gp32) and an immunity repressor (gp70) while Lewan contains a tyrosine integrase gene (gp38), immunity repressor (gp40), Cro (gp41), and excisionase (gp42), all suggesting possibly lysogeny. Future research will investigate the temperate nature of these two mycobacteriophages.

# 11th Annual SEA Symposium Abstract

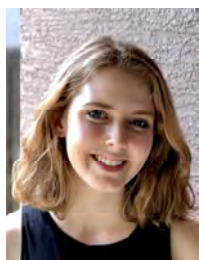
University of Alabama at Birmingham

Birmingham AL

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



**Rose M Albert**



**Carly R Snidow**

## ***Immunity Testing as a Probe for Phage Diversity Prior to Full-Genome Sequencing***

**Rose M Albert**, Alec Ayala, Mia S Bradley, Reuben E Burch, Mandy Chen, Anna Grace Dulaney, Pranav S Kakulamurri, Kelly U Kim, Seth D Maynor, Areebah I Nur, Sarah E Perritt, Hrithik Praveen, Dylan M Slemmons, **Carly R Snidow**, Sasha Thalluri, Atharva K Vyawahare, Mckinley R Williams, Denise L Monti

Bacteriophages follow one of two distinct life cycles upon infection of a permissible bacterial host. Lytic phages inject phage DNA into the bacteria and hijack the metabolic systems of the host to make new viral particles. The release of progeny viral particles causes the host cell to die which results in the presence of visible plaques on a lawn of host bacteria. In contrast, temperate phages infect the host and the phage genome integrates into the host bacteria genome via use of the phage integrase. Subsequent suppression of phage gene transcription is mediated by the cognate phage repressor. Each time the bacteria host (now called a lysogen) replicates, the integrated phage genome (prophage) is replicated along with the host. Under certain conditions, the phage genome excises from the host and the phage enters into the lytic cycle which often results in turbid plaques on a bacteria lawn. In 2017, students at UAB isolated five new *Corynebacterium* phages infecting the host *C. xerosis* (Juicebox, KobeBeanBryant, StAB, SamW, Troy). Stable lysogens were isolated from all five phages and cross-infection studies showed that phages with similar genomes (SamW and Troy) were homoimmune, while phages with dissimilar genomes (SamW and KobeBeanBryant) were heteroimmune. In fall 2018, we isolated 17 additional phages infecting the host *C. xerosis*. Preliminary genome characterization using restriction endonuclease digestion showed unique banding patterns for many of the phages so immunity testing using the 5 lysogens from 2017 was used as a secondary criteria for

sequencing selection. Five phages (Adelaide, Bran, Dina, Lederberg, Stiles) were selected for full-genome sequencing and annotated in spring of 2019. Four of the five sequenced 2018 *C.xerosis* phages contained a tyrosine integrase gene indicating most *C.xerosis* phages may be temperate. Thus, we sought to isolate stable lysogens for each of the 17 newly isolated *C. xerosis* phages and completed additional immunity experiments to further probe the diversity of *C.xerosis* phages prior to full genome sequencing.



# 11th Annual SEA Symposium Abstract

University of California, Los Angeles

Los Angeles CA

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



**Andrew Kapinos**

## ***Comparative Genomic Analysis of 31 Siphoviridae Reveals Extensive Host-Dependent Relationships and Novel Phage Subclusters***

**Andrew Kapinos**, Lauren Remijas, Nina Canela Torres, Ryan Ngo, Kris Reddi, Amanda Freise, Jordan Moberg Parker

Bacteriophages comprise the most abundant group of biological entities on the planet. As a ubiquitous feature of the Earth's various ecosystems, this class of organisms is estimated to outnumber all other organisms on the planet combined. If we are to fully harness the current momentum in phage research, we must continue to investigate evolutionary relatedness among phages. In this study, we hypothesized that host evolutionary processes may govern patterns in phage relatedness; as such, we predicted that phage genetic similarities resulting from coevolutionary pressures would reveal the intricate relationships which exist among phages, allowing for improved understanding of the bacteriophage pangenome. In our preliminary analysis, the *Gordonia terrae*-infecting phage Tanis was selected for genome annotation. Phage Tanis is a member of phage cluster DJ, a relatively new addition to the phage clustering system: of the 14 member phages that have been discovered since cluster DJ's inception, 50% have been isolated in the past year alone. Thus, it is unsurprising that 76.9% of genes identified during manual annotation possessed no known function. Comparative genomic analyses were performed using 21 additional *Siphoviridae* and their hosts (phages were isolated on hosts belonging to 2 phyla, 3 genera, and 11 species of bacteria). Phage and host phylogenies confirmed expected patterns of phage- and host-relatedness. Codon usage analyses revealed that phages with similar codon biases tended to infect hosts belonging to the same genera. Dot plots illustrating phage nucleotide similarity also revealed extensive regions of conserved nucleotide content, with increased statistical noise among phages infecting the same host; specifically, Actinobacteriophages exhibited genetic similarities distinct from other phage groups. Trends in average nucleotide identity (ANI) further supported the relationships observed during phage dot plot analyses; however, various patterns in ANI values challenged the current organization of several phage clusters, prompting an expanded investigation which included all known cluster DJ phages. Notably, our expanded ANI and core gene analyses provided evidence for the subdivision of cluster DJ, potentially calling for the creation of up to 4 novel DJ subclusters. Overall, the predominance of genes with unknown function in the Tanis genome demonstrated the need for continued research on phage biology. Initial comparative analyses supported a mechanism for phage-host coevolution, wherein the evolutionary distance between phages was directly proportional to the evolutionary distance between hosts. ANI analysis supported qualitative measures of phage relatedness and suggested the reorganization of cluster DJ phages

into discrete subclusters. In sum, our individual and comparative genomic analyses provided insight into the complexity of this growing field, while demonstrating the need for ongoing reevaluations of phage relatedness.

# 11th Annual SEA Symposium Abstract

University of California, San Diego

San Diego CA

Corresponding Faculty Member: Rachel Dutton (rjdutton@ucsd.edu)



**Avani Mylvara**

## ***Isolation and Characterization of A0 subcluster Arthrobacteriophage King2 and BK1 subcluster Streptomyces bacteriophage Beuffert from soil samples in San Diego County***

**Avani Mylvara**, Isaac Chang, Samantha Tuohey, Lauryn Reugg, Chaylin VanDenburgh, Tracy Le, Madeline Dunsmore, Liliana Zamora, Paty Esquer-Romero, Jennifer Park, Jeffrey Keller, Alison Washburn, Patrick Akarapimand, Grace Kim, Ceasar A De La Fuente, Emily Morton, Calvin Ha, Andrew Mueller, Lena Krockenberger, Adrienne N Santa Romana, Lou Devanneaux, Brian Khov, Taylor Lichtenberg, Thisha Thiagarajan, Lydia Irons, Victoria Ortiz, Samantha Licon, Rachel Dutton, Swarna Mohan

In the Phage Genomics Research initiative course at UCSD, students isolated 9 *Streptomyces platensis* phages from soil samples and another 9 *Arthrobacter* sp. phages. The phages were characterized after direct isolation by restriction enzyme digestion and gel electrophoresis.

Genomes of one *Arthrobacteriophage*, King2, and one *Streptomyces* phage, Beuffert, were sent to the Pittsburgh Bacteriophage Institute for sequencing. Phage King2, a lytic myoviridae phage, belongs to subcluster AO1, all of which only infect *Arthrobacter* sp., strain ATCC 21022. King2 and has 75 genes in 50,000 base pairs. 30 of these genes had known functions while the remaining 60% of genes had unknown functions; King 2 does not encode for any tRNAs.

Phage Beuffert, a lytic siphoviridae phage, belongs to subcluster BK1 and infects *Streptomyces platensis* MJ1A1. Beuffert has 130,000 base pairs with 238 genes and GC content of 47.7%. 39 of these genes had known functions, making 83.6% of gene functions unknown. Beuffert also encodes for 34 tRNAs. Comparisons of Beuffert and King2 to other phages in their subclusters using BLAST and Phamerator revealed several highly conserved genes and potential functions. Our research revealed substantial diversity in the final draft annotation of these phages' genomes by identifying genes and comprehensively examining their functions. Our annotations can benefit other researchers in determining further unknown, novel gene functions in similar phages and can benefit other researchers in finding new uses for such phages, like phage therapy.

# 11th Annual SEA Symposium Abstract

University of Central Oklahoma  
Edmond OK

Corresponding Faculty Member: Hari Kotturi (hkotturi@uco.edu)

## ***Alyxandracam: A Newly Isolated and Annotated Microbacteriophage from Oklahoma Soil***

Cameron Kedy, Alyxandra Siemer, Destinee Wilkins, Hari Kotturi

Bacteriophages are viruses which selectively invade bacterial host cells, taking control of the enzymatic machinery associated with replication and subsequently multiplying within the host. Throughout this project, our goals were to isolate a microbacteriophage from Oklahoma soil, characterize it, sequence and annotate its genome. The microbacteriophage Alyxandracam was isolated from reddish-brown clay soil located in a field near a residential area. We used the direct isolation method for isolating the phage and did three rounds of plaque purifications to obtain a pure phage. We used webbed plates for obtaining a  $2.3 \times 10^{10}$  pfu/mL high titer concentration, and transmission electron microscopy revealed a siphoviridae morphology. Phage DNA was extracted using the PCI method and was sequenced via Illumina sequencing technology. These methods enabled us to isolate, characterize and sequence a microbacteriophage from Oklahoma soil successfully. Bioinformatics tools were used to annotate Alyxandracam's genome. Our phage has a circularly-permuted, 41,770 bp genome with 63.4% GC content. Alyxandracam belongs to cluster EA and subcluster EA1, and appears to enact a lytic viral cycle. Alyxandracam has 63 genes, 25 of which appear to have known protein function based on BlastP and HHPred comparison. No tRNAs were found, and there did not appear to be a translational frameshift within the tail assembly chaperone genes.

# 11th Annual SEA Symposium Abstract

University of Colorado at Boulder  
Boulder CO

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



**Daniel Feeny**

## ***Characterizing Genetic Elements in the Cluster O Mycobacteriophage Blessica***

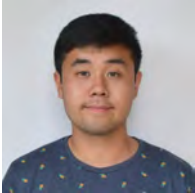
**Daniel Feeny**, Daria Nicke, Jack Carter, Luis Deschamps, Sarah Grout, Alex Haberman, Caleb Hanson, Jordan Kassanoff, Sophie Nabb, Daniel Petite, Gregory Prah, Zachary Rotblatt, Ojaswi Uprety, Josh Wild, Nancy Guild, Christy Fillman

Cluster O bacteriophage genomes are highly conserved and contain several characterized genetic elements including a MPME (Mycobacteriophage Mobile Element), inverted repeat sequences, and SigA promoter sequences. In this study we compared a new cluster O phage, Blessica, to other cluster O mycobacteriophages and identified these sequence elements, which provided insight into the evolutionary history of these phages.

# 11th Annual SEA Symposium Abstract

University of Connecticut  
Storrs CT

Corresponding Faculty Member: Simon White (simon.white@uconn.edu)



Tony Li

## ***Using Maximum Likelihood Phylogenetic Trees to Assess Horizontal Gene Transfer in Bacteriophages and Archaeal Viruses***

Tony Li

Bacteriophage evolutionary biology has always been difficult to study because of the recombination and exchanging of genes. Using data from PhagesDB, maximum likelihood phylogenetic trees of the major capsid protein and a non-structural protein, the viral DNA polymerase, were constructed and compared. With multiple well supported clades, one specific clade's movement indicates a possible horizontal gene transfer event while the rest show no sign of gene transfer.

During the annotation of our phage genomes, we found multiple surprising BLAST results matching a dsDNA phage with Haloviruses, which prompted an investigation of gene transfer between the dsDNA bacteriophages and Haloviruses. Their trees, though not as strongly supported, indicated much more movement within families of phages with homologous proteins than would be expected by horizontal gene transfer.

# 11th Annual SEA Symposium Abstract

University of Detroit Mercy  
Detroit MI

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



**Demitra Loucopoulos**

## ***Annotation of the M. foliorum specific EA1 Bacteriophage Ioannes and Zada***

**Demitra Loucopoulos**, Andrea Sandoval, Akhteyar Jaeran, Ali Zamat, Ken Dada, Jayla Anderson, Tania Anderson, Michael Avalos, Adrian Avila, Sheku Bangurah, Kiefer Degener, Razan El Yaman, Mohammad Hadeed, Leen Issa, Katelynn Kowalski, Yamere Lloyd, Vanessa Manzo, Nicolas Nunez, Semaj Shelton, Steven Taddei, Jonathan Finkel, Stephanie Conant, Jacob Kagey

Two *Microbacterium foliorum* specific bacteriophage were isolated from Michigan soil samples and purified. DNA was extracted and sent to the University of Pittsburg for sequencing, followed by genome annotation by the 2018-2019 SEAPHAGE course at the University of Detroit Mercy. The phage annotated were Ioannes and Zada, both EA, subcluster EA1 members. The EA1 subcluster has only 64 total phage identified and annotated thus far specific to the *M. foliorum* host as a part of the HHMI SEAPHAGE consortium. Annotations of these phage genomes identified gene mis-calls, insertions, false positive and false negatives within each genome. Because of the close similarity of the Ioannes and Zada genomes, phylogenetic comparison of Ioannes and Zada with other EA1 subcluster members was performed to investigate conserved genome similarities and protein-folding assessment was performed to identify potential differences resulting from minor nucleotide sequence changes.

# 11th Annual SEA Symposium Abstract

University of Evansville

Evansville IN

Corresponding Faculty Member: Ann Powell (ap96@evansville.edu)

## ***A Comparison of Three Subcluster BE1 Phage***

Michael Anderson, Katelyn Brown, Justin Cummings, Zach Eaglin, Sarah Kempf, **Anthony Kluemper**, James Pearce, Storm Poser, Darcie Smith, Spencer Thompson, Joyce Stamm, Ann Powell

Annotation of three subcluster BE1 genomes, Evy, Daubenski, and Braelyn, will lead to a better understanding of genome structure in *Streptomyces* phage. Genomes were annotated using PECAAN and functions were assigned using PhagesDB BLASTp, NCBI BLASTp, and HHpred. tRNA and tmRNA were identified and trimmed using Aragorn 1.2.38 and tRNAscanSE. Genome size in these phage ranged from 131,234 to 133,090 bp and annotations identified between 218 and 226 genes. Gene order, genome size, number of genes, and tRNA number and position match other subcluster BE1 phage. The three genomes also contain the large terminal repeats of about 10,000bp that are characteristic of subcluster BE1 genomes. Putative RIIA and RIIB-like genes were identified in all three genomes and this function was supported by NCBI BLASTp and HHpred comparisons.



# 11th Annual SEA Symposium Abstract

University of Hawaii at Manoa  
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**Chloe G Severson**

## ***Determining the Function of the Hypothetical Protein Encoded By Gene Twenty-Two of the Makai Bacteriophage Genome***

**Chloe G Severson**, Isabella AR Margerin, Chloe L Moore, Andrew R Barrows, Jake W Franco, Chelsee J Javier, Kimberly A Lucero, Elizabeth R Madrid, Kennedy S Neustel, Kamalani Oshiro, Nainoa W Ornellas, Abby Wilcer, Lauren H Vavra, Stuart P Donachie, Megan L Porter, Floyd A Reed, Sitara Palecanda, Rebecca A Chong

Of the eighty-nine genes in the sequenced Makai bacteriophage genome, we used BLAST and HHpred to define sixty-four genes as coding for the function of “hypothetical proteins”. Due to the abundance of these proteins with unknown functions, we decided to investigate the function of one of these hypothetical proteins. After running the amino acid sequences of identified “hypothetical proteins” through SWISS-MODEL, we chose to investigate gene twenty-two due to its complex protein structure. SWISS-MODEL also defined the protein’s local and global quality estimates, coverage, and possible ligands. We ran the amino acid sequence through protein structure prediction programs InterPro, Phyre2, and ProFunc. We then identified domains, repeats, detailed signature matches, predicted GO terms, cellular components, and identified portions of gene twenty-two for which one protein function had been determined with 99.9%-100% confidence. Using these tools, we determined that the product of gene twenty-two in the Makai genome is involved in general catalytic activity as a function of hydrolase enzymes. Evidence supports that the function of gene twenty-two’s product in the Makai bacteriophage genome is that of a hydrolase enzyme. Based on the data available for other bacteriophages, we determined that this catalytic activity involving hydrolase enzymes occurs in the tail region of bacteriophage Makai and aids in the transference of DNA between virus and host.

# 11th Annual SEA Symposium Abstract

University of Ibadan

Ibadan

Corresponding Faculty Member: Olubusuyi Moses Adewumi (adewumi1@hotmail.com)

## ***Cluster FE-like Bacteriophages infect a range of Bacteria and can be lysogenic***

Tolulope J Oduselu, Anjolaoluwa E Taiwo, Ifeoluwa E Ayodele, Oluwatimileyin T Oyebamiji, Nanayaa A Atoyebi, Modupeoluwa C Omolola, Uchemadu F Lazarus, Adefola L Jose, Elijah M Akinlolu, Babatunde M Ojebola, Olakunle S Olatinwo, Muideen K Raifu, Ini Adebisi, Olatimbo V Ogunleye, Temitope O Faleye, Adeleye S Bakarey, Olubusuyi M Adewumi, John I Anetor, Olusegun O Ademowo

Actinobacteria is a phylum of high G+C Gram positive bacteria with several members (e.g. *Mycobacterium tuberculosis*; the etiologic agent of TB) of economic importance. The recent upsurge in isolation and characterization of Phages of Actinobacteria (Actinophages) is courtesy the SEA-PHAGES program and has resulted in the isolation of over 15,000 Actinophages distributed into over 120 clusters, with the genomes of over 2,000 already deposited in GenBank. Only three genomes exist till date for Cluster FE and are all from lytic phages. In our effort to annotate Idaho (a member of the cluster), we stumbled onto anecdotal evidence that Cluster FE phages lysogenize. Here we describe our findings.

Identification of sequences of putative Cluster FE Actinophage-related prophages was done by BLASTp search of predicted Cluster FE phage proteins against the non-redundant protein database at NCBI. The Portal protein (PP), Major Capsid and Protease fusion Protein (MCPFP) and Tape Measure Protein (TMP) sequences of Cluster FE Actinophages were used as queries in the searches with default parameters. Recovered genomes were further screened (using Artemis) for low GC islands that correlated with coordinates from the BLASTp search. Confirmed low GC islands with Phage coding regions were then extracted. Subsequently, prophages were independently auto-annotated using both DNAMASTER and the RAST server and annotations were visually screened using both DNAMASTER and Artemis. Genome Maps were subsequently generated using DNAMASTER. Amino acid similarity of the PP, MCPFP and TMP proteins was done using MEGA5. The structure of TMP in all the ProPhages was predicted using ITASSER.

Significant hits spanning entire genomes were recovered from five Actinobacteria genomes belonging to genus *Cellulosimicrobium* (2 prophages), *Arthrobacter* (3 prophages) and *Microbacterium* (1 prophage). Genome Size ranged from 12.5kbp-16.5kbp. GC content ranged from 56.2%-70.5%. Genome organization was very similar with structural genes in synteny and same with non-structural genes. Number of genes per genomes varied from 16-23. Across genes and genomes, overall nucleic acid sequence similarity was extremely low. Amino acid similarity was also very low but higher than nucleic acid similarity. Despite significant amino acid diversity, the TMP structure was absolutely conserved across all the genomes.

We describe six FE Cluster prophages in Actinobacteria genomes as evidence that these Phages infect a range of Actinobacteria and have a lysogenic phase in their life cycle. We further show that though the genome organization and protein structure of Cluster FE phages is relatively conserved, the sequence similarity, genome size and number of genes vary.

# 11th Annual SEA Symposium Abstract

University of Ibadan

Ibadan

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**Olusegun G Ademowo**

## ***SEA-PHAGES 2018-2019 Session: The Ibadan Phage Hunters Experience***

**Olusegun G Ademowo**, John I Anetor, Olubusuyi M Adewumi, Temitope O Faleye, Adeleye S Bakarey, Olatimbo V Ogunleye, Ini Adebiji, Muideen K Raifu, Tolulope J Oduselu, Anjolaoluwa E Taiwo, Ifeoluwa E Ayodele, Oluwatimileyin T Oyebamiji, Nanayaa A Atoyebi, Modupeoluwa C Omolola, Uchemadu F Lazarus, Adefola L Jose, Elijah M Akinlolu, Babatunde M Ojebola, Olakunle S Olatinwo

The University of Ibadan SEA-PHAGES team comprise of seasoned faculty members and selected 300 Level Biomedical Laboratory Science undergraduates. The 2018 rookies embarked on a quest to isolate and identify soil borne actinobacteriophages in the city of Ibadan. This attempt was however hampered by constraints ranging from unavailability of isolation host, limited resources and disrupted academic calendar. These constraints limited our capacity to isolate and characterize indigenous bacteriophages during the discovery undergraduate research course offered in first semester. Nevertheless, during the genome annotation and bioinformatic analyses second semester course we maximized the opportunity presented during the December 2018 Bioinformatics meeting. Knowledge gained at the meeting was imparted into our students and we subsequently requested to annotate previously isolated Idaho Phage.

Within a short time, Idaho, a member of Cluster FE with a genome length of 15675 and an overhang sequence of 15 bases and a total of 22 genome features was annotated by the team. It was a great opportunity to train and see our undergraduates develop skills and show mastery in the use of variety of bioinformatic tools for genome annotation. It is worth mentioning that in the course of annotation, we noticed that Cluster FE-like bacteriophages including Idaho can be lysogenic and consequently showed 5 prophages with significant level of identity in genome organization (Oduselu et al., 2019). Ultimately, we succeeded in our scientific quest and today, with the assistance of the HHMI team, the annotated Idaho phage has been submitted to GenBank under the accession number MK757448.

Going forward, the University of Ibadan SEA PHAGES team, with institutional support and cooperation of our collaborators plan to annotate more bacteriophages, further explore our prophages experience, and more importantly isolate and characterize indigenous bacteriophages from our region.

# 11th Annual SEA Symposium Abstract

University of Kansas

Lawrence KS

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



**Sonja Jost**



**Zoey Manz**

## ***Isolation and characterization of CV-cluster *Gordonia* bacteriophages including JasperJr***

**Sonja Jost, Zoey Manz, Caleb Calhoun, Alexi Campidilli, Lexus Majors, Regan Putnam, Carlee Roberts, Shaelyn Marr, Avery Roe, Sharyn Sebert, Robert Ward**

The *Gordonia* phage JasperJr was isolated from a soil sample near the Pearson Scholarship Hall on the campus of the University of Kansas in the fall of 2018. Plaque morphology indicated that JasperJr is a temperate phage, and this was confirmed by making a JasperJr lysogen strain of *Gordonia terrae* in 2019. Sequence analysis revealed that JasperJr is a member of cluster CV. The CV Cluster is mostly composed of temperate phages, however, 8 different phams classified as immunity repressors have been found in the 29 CV cluster phages. This raised the question of whether CV phages should be clustered based only upon whole genome sequence similarity or whether CV should be subclustered based upon immunity. We therefore obtained additional CV phages, made lysogens of the CV phage Carol Ann and the CY phage Pollux, and tested immunity of these lysogens to a host of different temperate and lytic phages. Interestingly, CV cluster phages have been shown to possess genes similar in structure to those coding for prophage-mediated defense systems in N cluster mycobacteriophages. Though the functions of these CV cluster genes are poorly understood, their study may provide valuable insight into phage defense systems. Thus targeting CV cluster phages for isolation would be a convenient way to find additional genes for study. Using Phamerator, we found 4 genes that are highly enriched in CV cluster phages and not found in other clusters. We designed PCR primers to amplify regions between genes 12 and 13 and between genes 26 and 27 from JasperJr (and related phages). We found that these primer sets efficiently amplified CV cluster phages but not phages from a variety of other clusters. We could identify CV phages from a mixed phage sample having  $10^5$  excess of non-CV phages. We are attempting

to develop a protocol to use PCR to identify CV phages in enriched soil samples without filtering the enrichment, thereby saving time and money. Finally, we were interested in identifying the JasperJr proteins that were included in the infectious phage particles. We expected capsid, connector and tail proteins, but hoped to identify if any of the proteins with unknown functions were also included. Ultimately, we had hoped to use mass spectrometry, but were unable to get that far this semester. We did, however, make a large volume of JasperJr high titer lysate that we purified through a cesium chloride ultracentrifuge gradient to obtain a pure phage sample. We used polyacrylamide gel electrophoresis to separate phage proteins and visualized them using Coomassie and silver staining techniques.

# 11th Annual SEA Symposium Abstract

University of Lagos

Lagos

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## ***Big3, a close relative of MrGordo***

Emmanuella Omolade, Oyindamola Akinyemi, Kafayat Razaq, Taiwo Ogunfile, Blessing Omolere, Maryam Saliu, **Habeeb Aderolu**, Nzube Ekpunobi, Seun Bamisaye, Ayodeji Odunsi, Imade Nsa, Tenny Egwuatu, Ganiyu Oyetibo, Matthew Ilori

Big3 is a siphoviridae phage isolated from an enriched soil sample in Richmond, VA by Brandon Brown-Ruffin at Virginia Union University in 2016. Its genome was found to be 53442 bp in length and assigned to Cluster A, subcluster A1. Programs used for annotation include DNA Master, NCBI BLAST Phamerator, Starterator, GeneMark, HHPred and Gene Content Similarity (GCS) Tool. On annotation, we found 91 called ORFs, 49 had known functions, the expected mosaic patterns in the gene order of sub-cluster A1, and no tRNAs. About 56% were reverse genes, no orpham genes, 26% of the start sites began with GTG, 5.5% with TTG and others with ATG. Big3's annotation revealed a very strong resemblance to another temperate phage MrGordo isolated by Sean Kearney at Purdue University. Using the GCS tool, Seventy-eight phams were common to both with a GCS of 85.72% and fifty out of the 78 shared phams had the exact same ORF length. They were identical in their gene sizes of their tape measure, Lysin B, portal, capsid maturation protease, integrase, endonuclease VII proteins to name a few. They had the exact order of gene functions including the programmed translational frameshift at the tail assembly chaperone except that MrGordo had a split primase, only one terminase, more structural proteins but no superinfection immunity and helix-turn-helix binding domain proteins. On the other hand, Big3 had more minor tail proteins, small and large terminase subunits and membrane proteins. In addition, the largest ORF in Big3 was the large terminase subunit (2712 bp) followed by the tape measure protein (2472 bp), whereas, for MrGordo, tape measure (2472 bp) was the largest.

# 11th Annual SEA Symposium Abstract

University of Louisiana at Monroe  
Monroe LA

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## ***Comparison of the *Gordonia terrae* CU3 Cluster Phage Dardanus with CU1 Cluster Members and the Singleton Phage Catfish***

**Breanna A Gottschalck**, Hart A Bordelon, Ethan M Brister, Amanda M Bryans, Ashlynn E Calk, Christian C Capers, Jacob M Corrent, Cecil N Delphin, Grant W Erbeling, Brandon T Hale, Nautica T Jones, Angelle R Mire, Austin R Perkins, Rebecca D Quackenbush, Chelsi S Rogers, Nicholette C Stewart, Hannah N Threeton, Zachary F Wiggins, Anne Marie Hancock, Aaron V Nguyen, Allison M D Wiedemeier, Christopher R Gissendanner, Ann M Findley

During the 2017-18 academic year, ULM freshmen SEA PHAGES Program students isolated 24 novel bacteriophages using the *Gordonia terrae* host system. *Gordonia* phage Catfish was sequenced and determined to be a Singleton with no clear homology to any previously sequenced *Gordonia* phage. Catfish has 46,888 bp with a 3' sticky overhang of eleven bases, 79 open reading frames, and a GC content of 65%. In functionally annotating the Catfish genome, highest BLAST hits for the entire genome and many of its functional open reading frames (ORFs) corresponded to members of the CU1 cluster of *Gordonia terrae* phages. We have since adopted *Gordonia* phage Dardanus for annotation (isolated by Florida Gulf Coast University). Dardanus, the sole member of the CU3 cluster of currently-sequenced *Gordonia* phages, has 43,143 bp, 74 ORFs, no tRNA genes, a GC content of 66%, and shares many of its functional ORFs with members of the CU1 cluster and Catfish. We provide a functional annotation of Dardanus and explore its relationship to CU1 cluster *Gordonia* phages and the Catfish singleton using the SplitsTree, Gepard DotPlot, and Phamerator visualization tools. Such analyses provide insight not only into the relationship between the *Gordonia terrae* phages but can point to extended comparisons between other Actinobacter phage group isolates.

# 11th Annual SEA Symposium Abstract

University of Maine, Farmington  
Farmington ME

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**Mikahla Searles**

## ***Discovery and analysis of Subcluter DE3 phage RoadKill from a wastewater treatment facility***

Mikahla Searles, Naomi Moulton, Liz Nadeau, Paige Ring, Jean A Doty, Timothy S Breton

Bacteriophages are useful tools in a variety of biomedical and molecular research studies, including efforts to combat antibiotic resistance and understand both DNA recombination and protein function. A novel bacteriophage able to infect *Gordonia terrae* was isolated from a wastewater treatment facility in Farmington, Maine and named Roadkill. Roadkill is a siphoviridae phage that produced clear, round plaques and is member of Subcluster DE3. RoadKill's genome is circularly permuted and consists of 55,939 bp and 84 genes. Similar to other Cluster DE phages, the RoadKill genome does not contain any well-established genes associated with lysogeny, and may only exhibit lytic growth. RoadKill is highly similar to other DE3 phages (96-97% identity) but also exhibits some novel recombination, including after the terminase gene and between genes 65 and 68. These four genes were possibly inserted into a protein coding sequence with high similarity to orpham gene 69 in phage Dxdert. The central region of this protein in Dxdert (residues 104-141) exhibits similarity to the end of orpham gp69 in RoadKill. In addition, the same residue (104) and terminal end of the protein is associated with high similarity to orpham gp73 in phage GTE6. Overall, this genomic location in the DE3 phage genes may be associated with high rates of insertion and may require further investigation.



# 11th Annual SEA Symposium Abstract

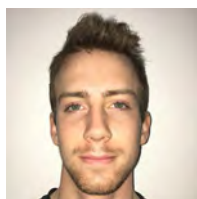
University of Maine, Honors College

Orono ME

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**Jessica Hayden**



**Andrew Fournier**

## ***A study of phage with attitudes: defensive *Gordonia* phage Sidious and MagicMan and crazy *Rhodococcus* phage Whack and SleepyHead***

**Jessica Hayden**, Emmie Baillargeon, Alec Ballinger, Alex Barrios, Jack Beaulieu, Angela Burke, Jack Burnell, Jacob Cote, Matthew Cox, Morgan Crapo, Samuel Deelsnyder, Hope Dorr, Trevor Dugal, **Andrew Fournier**, Caiden Fraiser, Remi Geohegan, Benjamin Gove, Keegan Gray, Hanna Griffin, Kaitlyn Jodoin, Nate Jordan, Marissa Kinney, Sarah Latario, Sarah Low, Victoria Mayers, Kyle Murawski, Eva Nazim, Erika Pacheco, Jenn Quezada-Loja, Zeke Robinson, Matt Seuch, Gabby Spencer, Dylan Taplin, Marc Thibodeau, Zach Williams, Ryan Worthington, Melissa Maginnis, Melody Neely, Sally Molloy

Through use of multiple bacterial hosts in the SEA PHAGES classroom, UMHC, isolated and sequenced two singleton *Rhodococcus* phage and six novel *Gordonia* phage belonging to clusters A15, CT, CZ7, DB, DC, and DU. Two of the *Gordonia* phage, Sidious and MagicMan, are temperate and encode viral defense systems. Sidious is the sole member of subcluster CZ7. Its genome is 51,789 bp in length, has a GC content of 66.6%, and encodes 84 putative genes. Sidious shares an immunity repressor with cluster CZ1 phage BatStarr and Nymphadora and *G. terrae* lysogens of Sidious are homoimmune with these phage. Sidious lysogens are also immune to infection by phage that do not have related immunity repressors: Yeezy and BaxterFox (CZ3); BetterKatz (DI) and Sitar (DE1). This heterotypic immunity may be due to Sidious genes gp41 and 40, which encode a putative abortive infection system, RexA and RexB, respectively. MagicMan is a cluster DB phage with a 47,598 bp genome with 67% GC content. The genome encodes 70 putative genes including an integrase (gp37), immunity repressor (gp38), and putative BrnT-like toxin (gp35). An obvious anti-toxin gene was not identified; however gp36 is divergently transcribed relative to gp35 and the gene product has a C-terminal ribbon-helix-helix domain, consistent with the antitoxin BrnA. *Rhodococcus* phage SleepyHead and Whack are temperate, singleton phage. The SleepyHead genome is 43,943 bp in length, has 61% GC content, and encodes 67 putative genes, including 37 orphans. SleepyHead encodes an immunity cassette that includes a reverse oriented immunity repressor (gp40), a peptidase (gp39) and tyrosine integrase (gp38). The peptidase has a strong HHPRED match to ImmA Zn-dependent peptidases and could function as an anti-repressor. There are 11

reverse genes between the integrase and the minor tail proteins that include 2 transposases, a ribonuclease and a membrane protein. *Rhodococcus* phage Whack has a 49,660-bp genome with 61.9% CGC content. Whack is also a singleton, sharing only 25% of its 77 putative genes with its closest relative, singleton *Rhodococcus* phage, REQ2. Nearly half of Whack's genes (43%) belong to orphans, including the immunity repressor and tyrosine integrase. Downstream of the integrase are 7 reverse oriented genes that include 3 DNA binding proteins, 2 membrane proteins and a lipoprotein.

# 11th Annual SEA Symposium Abstract

University of Mary  
Bismarck ND

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**Lorren Postl**

## ***Host range infectivity studies of three unique annotated phages, Jiminy (B1), Doug (F1) and Malthus (K4) discovered in water and soil samples near Bismarck, ND***

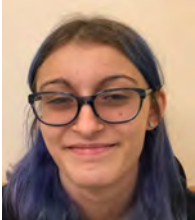
**Lorren Postl**, Benjamin Ahlbrecht, Heidi Bau, Hope Bodnar, Casi Boehm, Zoe Fath, Madison Holm, Marina Lula, Kylie Pastian, Joey Popiel, Joel Weisbrod, Frances Whitman, Joseph Angstman, Grace Burns, Micah Zimmerman, Christine Fleischacker

The students in the Phage Discovery Research Course at the University of Mary discovered in the Fall of 2018, three novel phages using *Mycobacterium smegmatis* mc2 155 as the host cell. This is the 3rd year of being a part of the SEA Phages Program, which is sponsored by HHMI Science Education Division. The first phage, Jiminy, was isolated from a water sample from the Apple Creek near the University of Mary and is a B1 subcluster phage. The second phage, Doug, was isolated from the garden soil of the University's President and is an F1 subcluster phage. The third phage, Malthus was isolated from soil near the University and is a K4 subcluster phage. Electron microscopy revealed all three phages to belong to the phage group of siphoviridae. Jiminy had the largest genome with 68777 bp and GC content of 66.4%. Both Doug and Malthus had smaller genomes, 58397 bp and 57802 bp respectively and GC content of 61.1% and 67.9% respectively. Further investigation into the host range infection capabilities using different mycobacterium hosts for these phages revealed interesting results. The three phages, despite being from different subclusters, had varying abilities to infect multiple hosts. Their genomes were annotated in the Phage Genomic Analysis Research Course in the Spring of 2019 and the host range infectivity results as well as the comparison of their genomes will be discussed. These phages were tested using alternative mycobacterium hosts of *Mycobacterium smegmatis*, *Mycobacterium phlei*, *Mycobacterium nonchromogenicum*, *Mycobacterium fortuitum*, *Mycobacterium chelonae* and *Mycobacterium marinum*.

# 11th Annual SEA Symposium Abstract

University of Mary Washington  
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**Kayla N Botto**

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

**Kayla N Botto**, Joseph D Callery, Kimberly M Corbett, Pamela D Daniels, Kaylee D Deardorff, Julia K Gasink, Grace W Holcomb, Jack J Levine, Sean D Lockwood, Angus S Long, Madelynne E McIntosh, Gabrielle P Moore, Natalie J Mroz, Rina Murasaki, Kaitlyn O Parker, Ty G Stephenson, Bennet T Varghese, Theresa M Grana, Lynn O 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 17 phages isolated this year, two (Grumio and FreightTrain) were sequenced. Both were isolated from enriched cultures, both were myoviruses, and Grumio was found in soil from Woodbridge, VA, while FreightTrain was isolated from soil collected in Fredericksburg, VA. Grumio has a genome length of 161,495 bp, which autoannotated with 294 features, a direct terminal repeat of 2,585 bp, and a GC content of 38.7%. Grumio is most similar to Archie14, ALPS and Rex16 by BLAST. FreightTrain has a genome length of 162,107 bp, which autoannotated with 297 features, a direct terminal repeat of 2,823 bp and a GC content of 38.7% and is most similar to DIGNKC, Zuko and AaronPhages. Neither phage contained any tRNA genes when searched with tRNA Scan. A host range study was conducted with Grumio, demonstrating that it could infect both *B. subtilis* and *B. anthracis* Delta Sterne in addition to *B. thuringiensis*. Both phages belong to subcluster C1.

# 11th Annual SEA Symposium Abstract

University of Maryland, Baltimore County  
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**Michelle L Guldán**

## ***Of clusters and mischief: the *Streptomyces* phageome***

Kristina E Atanasoff, Ashley Batista, Courtney L Colson, Gregory Furletti, Stephen Goralski, **Michelle L Guldán**, Matthew S Kane, Jenni Kelleher, George Kyaw, Sarah V Lance, Dorjan Leka, Shirin Ludwig, Victoria Mahaney, Phoebe Mariano, Tanzeem Naqvi, Thomas O'Brien, Sana Rana, Chih Shiang K Shee, Andrew L Simpson, Shreya Singh, Ryland N Spence, Racheal A Spruill, Lowen E Wachhaus, Claudia L Xie, Ivan Erill, Steven M Caruso

The 2018 UMBC Phage Hunters successfully isolated, characterized and sequenced the genomes of six bacteriophages infecting two hosts: the phytopathogenic *Streptomyces scabiei* and heavy metal-tolerant *Streptomyces mirabilis*. Five of the phages are *Siphoviridae*, including: two BB2 phages, Heather and RemusLoopin; a BG phage, Mischief19; a BI2 phage, PherryCruz; and a singleton, RosaAsantewaa (accession numbers MK686069-MK686072). The sixth phage, Forthebois, is a *Tectiviridae* and is in cluster BO (accession number MK620900). Analysis of the annotated genomes with Phamerator, Geppard plots, and the Gene Content Similarity (GCS) metric revealed that the cluster assignment for some of these phages may need to be revised. For instance, RosaAsantewaa is currently classified as a singleton, but shares 60% GCS with subcluster BI2. Conversely, re-assigning Mischief19, currently annotated as a BG phage, as the first member of subcluster BG2 would significantly decrease the dispersion of GCS values within cluster BG. To gain further insight into the accuracy of these putative reassignments, we explored the use of two highly conserved structural genes (those coding for the tape measure and terminase proteins, often syntenic) for phylogeny-based cluster assignment. The tape measure protein (TMP) generates noisier Geppard plots than the terminase protein due to the presence of internal repeats, limiting its applicability for clustering purposes. Further analysis enabled us to identify the repeat motifs in several clusters, and phylogenetic analysis also confirmed that repeats have an impact on alignment and subsequent tree inference. Using the tree distance for clustering, we observe that the results essentially recapitulate the established clusters, with some notable exceptions. Most notably, the TMP of Mischief19 bears little resemblance to that of its assigned cluster, supporting again that Mischief19 should probably define its own subcluster BG2. This phage displays a large insert between its capsid and tail fiber genes and almost half its genome is composed of orphans. Our bioinformatic analysis also detected the presence of a predicted protein in RosaAsantewaa with a partial match to the coliphage superinfection exclusion protein B (PF14163). This predicted protein appears to be conserved among BI phages, which have been consistently annotated as lytic. Examination of the BB2 subcluster, including phages Heather and RemusLoopin, revealed a high degree of conservation in this subcluster and the likely acquisition of a *thyX* gene in one of the subcluster phages (Sebastisaurus). We discuss the possible role of superinfection exclusion proteins in lytic phages, and of thymidylate synthases in lysogenic ones.

# 11th Annual SEA Symposium Abstract

University of Nebraska-Lincoln  
Lincoln NE

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



**Phoebe B Peña**

## ***Isolation of phage that infect *Gordonia rubripertincta* and annotation of *Gibbous* and *Chikenjars****

**Phoebe B Peña**, Lindsay R Birge, Layton D Bivans, Seth M Blakestad, Emma K Chesley, Elizabeth E Frank, Sarah Hoagland, Justin Hultquist, Ethan P Ramsey, Nicholas R Lee

The *Gordonia* genus includes species capable of transforming and degrading hydrocarbons, making them candidates for environmental and industrial biotechnology (Arenskötter, et al., 2004). Phages of *Gordonia* are potential genetic tools that can be used to study the genes coding for the metabolic enzymes of the bacteria. To bolster the numbers of identified phage that infect the genus *Gordonia* (currently ~ 1600), students used *Gordonia rubripertincta* NRRL B-1654 as the host in the Fall 2018 Virus Hunting course at UNL. By direct isolation, six phage were successfully acquired, archived and added to The Actinobacteriophage Database (phagesDB.org). All six phage are siphoviridae as judged by their morphology viewed by transmission electron microscopy, having tails ranging in length from 200 to 250 nm. Two genome sequences, *Gibbous* (45,810 bp; 60.5% GC) and *Chikenjars* (61,544 bp; 51.3% GC) were determined by Illumina Sequencing by the Pittsburgh Bacteriophage Institute. Auto-annotation using DNA Master predicted 69 genes in *Gibbous* (cluster CT) and 95 genes in *Chikenjars* (cluster DJ). An obvious difference between the phage genomes, based on the auto-annotation, was the presence of 24 reverse ORFs in *Gibbous* clustered largely in the right arm of its genome, while *Chikenjars* has only four small reverse ORFs that are isolated and dispersed through the genome. Phamerator analyses showed that the two phages shared no Phams although both had the requisite known proteins of tailed phages including terminase, HNH endonuclease, portal protein, capsid maturation protease, scaffolding protein, major and minor capsid proteins, endolysins, major and minor tail proteins, and tape measure protein. Very close relatives of *Chikenjars*, phages Duffington and Rickmore, (cluster DJ), were isolated in Idaho using *Gordonia terrae* 3612 as the host, raising the interesting questions of whether they can infect *G. rubripertincta* and if *Chikenjars* can infect *G. terrae*. Similarly, phages Cozz and Emalyn (cluster CT), identified using *G. terrae* as the host, are relatives of *Gibbous*. The same question arises as to whether the phages can cross infect the other *Gordonia* species. Annotation by the class group found support for functional assignments of 31 out of 69 genes (45%) for *Gibbous*. For *Chikenjars*, 19 out of 95 ( 20%) of the genes had credible support for functional predictions.

# 11th Annual SEA Symposium Abstract

University of Nevada Las Vegas

Las Vegas NV

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Ryan Doss

## ***Cluster A1 and Cluster J Mycobacteriophages with Possible Homologous Putative Beta-Lactamase and Putative Superinfection Immunity Repressors***

Ryan Doss, Kurt Regner, Christy Strong, Philippos Tsourkas

We present the genomes of *Mycobacterium smegmatis* phages NihilNomen and Carlyle, isolated by students at the University of Nevada Las Vegas. The phages were isolated from compost in the University community garden. Both phages are temperate.

NihilNomen is a cluster J phage with a genome 110,439 base pairs long and GC content 60.8%, typical of cluster J phages. Its genome ends are 3' sticky overhangs 4 bp long. We identified 240 genes, including one tRNA.

Carlyle is a cluster A1 phage with a genome 51,220 base pairs long and GC content 63.6%, also typical of cluster A1 phages. Its genome ends are 3' sticky overhangs 10 bp long. We identified 91 genes in Carlyle, with no tRNAs. There was a population of unusual reads around position 29100 in Carlyle's genome, indicating that it could perhaps be two very closely related phages, or that there is a large mutant population in the sample.

NihilNomen appears to possess a third terminase (gp2), located upstream of the small and large terminase. It corresponds to what other researchers of cluster J phages report as a possible DNA-packaging protein.

NihilNomen also contains a putative pbp beta-lactamase gene at gp39 and a putative superinfection immunity repressor at gp196. These three genes will be investigated further during the summer.

In Carlyle, the small terminase (gp4) is not adjacent from the large terminase (gp13), with the lysin A (gp11) and lysin B (gp12) located in between. Carlyle also contains a putative pbp beta-lactamase gene (gp34), with 60% amino acid sequence identity match with the beta-lactamase in NihilNomen, as well as a putative superinfection immunity protein (gp74) with a 98% amino acid sequence identity match with that in NihilNomen. The putative beta-lactamase and superinfection immunity protein of Carlyle will be investigated this summer along with those of NihilNomen.

# 11th Annual SEA Symposium Abstract

University of North Georgia

Dahlonega GA

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**Kandice Cantrell**

## ***Environmental Effects on Mycobacteriophage Cepens - Host Interactions***

**Kandice Cantrell**, Ethan Strickland, Ryan Shanks, Alison Kanak

Bacteriophage (phage) are viruses that infect and use bacterial hosts for viral replication. Study of these bacterial viruses has led to such discoveries as knowing DNA is the molecule of inheritance. Study of phage has also led to discovery and implementation of novel therapeutic treatment of infections. Known as “phage therapy”, phage have been utilized in curing a patient of the highly virulent infection known as MRSA. Tuberculosis, caused by *Mycobacterium tuberculosis*, is a growing concern as more antibiotic-resistant strains become more prevalent in the population. Cepens is a lytic phage that infects members of the *Mycobacterium* genus. This study analyzes Cepens and its interactions with its host bacteria, *Mycobacterium smegmatis*, in an effort to understand how environmental conditions impact infection and replication efficiency. First, replication benchmarks were established by conducting a serial dilution and plaque assay under standard conditions. Baseline titer calculations were used for comparison after alteration of incubation temperature and pH of phage, both prior to and during infection. To determine the ability to withstand thermal exposure during infection, plated samples were incubated between 25°C-50°C. To test the effects of pH on infection, samples were titered on plates ranging from pH of 5.0-8.1. Thermal stability of phage lysate was determined after exposure to 37°C-65°C. Lastly, pH stability of phage lysate was also tested by exposing sample to a pH range of 1.0-10.0. Extreme temperatures and pH are expected to lead to a decrease in infectivity as well as potential degradation of the phage itself. The effect of variation in environmental conditions was shown through analysis of plaque presence, size, and number. Analysis of this data provides optimal conditions for maximum host infectivity and further contributes to the understanding of virus-host interactions.



# 11th Annual SEA Symposium Abstract

University of North Texas

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**Brendon Williams**

## ***Examination of Orphans following the Tape Measure of Streptomyces phage Celia, the first member of Subcluster BD6***

Brendon Williams, Julia Lopez, Katherine N Ball, Lee E Hughes

*Streptomyces* phage Celia was isolated on the host *Streptomyces xanthochromogenes* NRRL B-5410 in 2018 at the University of North Texas. Celia was sequenced at the University of Pittsburgh and found to be the first member of subcluster BD6. UNT students annotated the genome in the spring semester of 2019. Of Celia's 80 genes, 22 were found to be orphans, including a small region consisting of four genes in the region just after the tape measure gene. This region, which is expected to contain minor tail proteins, has been examined in other BD phages where a variety in both numbers of genes and the phams the genes belong to has been found. We are comparing Celia's genome with these other phages in order to better understand the implications of the orphans found in this region of Celia's genome.

# 11th Annual SEA Symposium Abstract

University of the Ozarks

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**Gracie J Millar**

## ***Identification and Annotation of Gordonia Phage Avazak***

Gracie J Millar, Adolfo Sotolongo

Antibiotic resistance in bacteria is a developing issue, that will continue to spread as bacteria adapt to current therapies. One possible solution to this issue is phage therapy. Phage therapy uses bacteriophages, viruses that only attack bacteria, to infect specific pathogenic bacteria. Recently, this has been used as a therapy of last resort. The demand for new phages and their classification has opened access to research and discovery for undergraduate students through the SEA-PHAGES program. Only a small percentage of estimated phages in our biosphere have been discovered and annotated. Researchers are striving to unearth more bacteriophages in order to understand their structure, function, ecology, and potential use in treating infections caused by antibiotic resistant bacteria. Through established protocols a phage, Avazak, was discovered, isolated, and its genome annotated. Avazak infects the bacterium *Gordonia rubripertincta*. *G. rubripertincta* is a soil bacterium of the phylum Actinobacteria and is gram-positive. Avazak is a cluster DJ phage which has 14 other known family members, who are Siphoviridae and have lytic life-cycles. Avazak forms small definite plaques and has 91 genes in its genome. Continued identification and characterization of novel bacteriophages will provide increased understanding of phages and may aid against antibiotic resistant bacteria.

# 11th Annual SEA Symposium Abstract

University of Pittsburgh

Pittsburgh PA

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**Swathi Tata**

## ***Investigating *Gordonia terrae* phages for the presence of prophage-mediated host defenses***

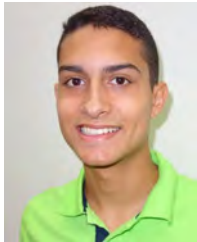
**Swathi Tata**, 2018-2019 Pitt SEA-PHAGES Course, Meghan Bechman, Rebecca Bortz, Kristen Butela, Brenda Hammer, Marcie Warner, Matthew Montgomery, Deborah Jacobs-Sera, Graham F Hatfull

The collection of over 15,000 actinobacteriophages, with 2,900 of these being sequenced, can reveal insights into phage diversity and evolution. The 2018-2019 student cohort of phagehunters at the University of Pittsburgh isolated 198 phages from environmental samples using the host *Gordonia terrae* 3612, contributing to the overall total of over 1,500 phages isolated on *Gordonia* hosts. The genomes of 19 of these phages were sequenced, adding to the existing collection of 379 sequenced *Gordonia* phages. These phage genomes are characteristically mosaic and span considerable genomic diversity, being assigned to 8 extant clusters (CS [4], CT [1], CV [1], CZ [5], DB [1], DC [2], DE [4], and DP [1]). One sample yielded two fully sequenced phages (JajaA [Cluster CV] and JajaB [Cluster CS3]) that we purified, separated, assigned to cluster using PCR, and annotated. Additional phages previously isolated by Pitt students in years 2016-2018 were annotated and assigned to 5 clusters (CV [2], CZ [3], DB [1], DC [1], and DN [2]). Phages isolated during the 18-19 academic year differ in genome length ranging from 46,096 bp (HannahD, Cluster DB) to 114,220 bp (Boopy, Cluster DS), and G+C% content, ranging from 50.1% (Ziko, Cluster DP) to 67.6% (Bakery, Cluster DC; *G. terrae* is 67.8%). Several temperate *G. terrae* phages in our collection display genetic mosaicism in the area immediately surrounding the immunity cassette, similar to what has been previously reported for the Cluster N mycobacteriophages. Such genes are candidates for novel prophage-mediated viral defense mechanisms. To investigate this possibility, previous students in the Pitt SEA-PHAGES course created a collection of *G. terrae* lysogens using Blueberry (Cluster CV), Utz (Cluster CV), UmaThurman (Cluster CV), Lilas (Cluster CY1), Vasanti (Cluster CZ2), Adora (Cluster CZ4), and Opie (Cluster DB). We tested a collection of over 100 sequenced and unsequenced phages for their ability to infect these lysogens, and preliminary data shows that these lysogens defend against infection from various phages, with plating efficiencies reduced by at least  $10^{-4}$  relative to the wild-type *G. terrae* host. Future directions include identifying candidate genes that could confer defense against superinfection in the lysogens we tested this year, generating lysogens from newly annotated temperate phages that show genetic mosaicism near the immunity cassette, sequencing the phages against which the various prophages tested here provide protection, and investigating lysogen gene expression and the phages they defend against.

# 11th Annual SEA Symposium Abstract

University of Puerto Rico at Cayey  
Cayey PR

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**Nayid E Jana-Martinez**

## ***Genomic comparisons of a set of diverse bacteriophages isolated from Puerto Rico***

**Nayid E Jana-Martinez**, Bonmarie Pedraza-Lopez, Denniel A Rosado-Nunez, Hector N Romero-Soto, Alondra Rivera-Cruz, Miosotty Aleman-Lozada, Fabiola Aponte-Olivieri, Ines N Aviles-Rivera, Hector Cartagena-Torres, Maria A Cruz-Velazquez, Samira J Diaz-Dingui, Bryan J Diaz-Melendez, Noelis Falcon-Lizardi, Nectar G Gonzalez-Negron, Karian A Martinez-Pagan, Valerie V Medina-Santiago, Andres S Millan-Laboy, Perla M Miranda-Melendez, Charlie Mercado-Mulero, Lizmary Morales-Diaz, Sophia L Negron-Caez, Robert J Nieves-Ayala, Maricelys Ortiz-Castro, Keishlean M Osorio-Medina, Kathya M Reyes-Laureano, Adriana Reyes-Soto, Yanidelis Rios-Rosa, Alondra Rivera-Cruz, Nicole S Rivera-Espinal, Edgar J Robles-Arocho, Fabian E Rodriguez-Cotto, Cynthia M Rodriguez-Cruz, Kevin I Rodriguez-Otero, Andrea N Rosa-Flores, Eliomar Rosa-Ramon, Janelly M Rosa-Vazquez, Gabriel Rosario-Ortiz, Alondra Rosario-Valdes, Gabriela V Seda-Alvarado, Juan A Vargas-Alvarez, Alondra Vargas-Lopez, Joe A Vazquez-Valentin, Roberto J Herrera-DelValle, Joelis M Lama-Diaz, Carlos E Ocasio-Caldero, Priamo A Pichardo-Gonzalez, Jose O Silva-Martinez, Mariceli Fernandez-Martinez, Michael R Rubin, Edwin Vazquez

The rich diversity of Puerto Rico's ecosystems is reflected in the variety of bacteriophages isolated during the ten years of participation of our institution in the SEA-PHAGES Program. We have isolated hundreds of different phages of which the genomes of 32 have been sequenced. These include twenty-one mycobacteriophages, ten *Gordonia terrae* phages, and one *Microbacterium* phage. We have streamlined the standard protocols to save time, effort and materials, without sacrificing the pedagogical aspects of the hands-on experience, and the deep-conceptual understanding of the biological processes involved. We now report on the isolation, characterization, genomic annotation and comparison of five novel *Gordonia* phages (Yndexa, Sukkupi, Syleon, MelBins and Keelan) and one *Microbacterium* phage (Zanella). We have compared their genomes in terms of the gene homology of various highly conserved regulatory and structural genes, and the divergence of others that have apparent essential functions but have probably not been submitted to the same natural selection pressures of the conserved ones. Among the genes studied are DNA-binding, tape measure and capsid proteins, and lysins. Three of the seven CR cluster *Gordonia* phages in PhagesDB are from Puerto Rico (BiPauneto, Sukkupi and Yndexa). Genome comparisons show that Sukkupi and Yndexa are highly similar, with a few sequence differences on both the left and right ends, indicating a very recent evolutionary divergence from a common ancestor. MelBins is a DE2 cluster phage and Keelan is from cluster DP, again

denoting the diversity of the phages isolated. Zanella, the *Microbacterium* phage, has the smallest genome size, at 42,108 bp, which is reflected in its short-predicted tail length based on the number of amino acids comprising the tape measure protein, at only 45 nm versus 325 nm for Syleon. Our results contribute to understanding the enormous genetic diversity of bacteriophages and the underlying similarities that point to their common evolutionary origin.

# 11th Annual SEA Symposium Abstract

University of the Sciences in Philadelphia  
Philadelphia PA

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**Colin Bokan**

## ***West Philadelphia Born and Phaged***

Sadia Ali, Carmen Biniek, **Colin Bokan**, Justin Derk, Allie Hushen, Zeina Issa, Jack Kisielius, Sarah Macko, Taylor Pompan, David Tomlinson, Abigail White, Dana Pape-Zambito

Bacteriophages are the most abundant organism in the biosphere. Approximately eighty percent of phage genomes lack known functions making phages prime candidates for experimental research. Furthermore, little information is available on bacteriophages that infect *Microbacterium foliorum*, thus this host was selected for bacteriophage isolation. Phage PhillyPhilly was recovered and isolated from a soil sample just outside the University of the Sciences in the heart of West Philadelphia. PhillyPhilly was sent to be sequenced within a DOGEMS pooled sample. Using PCR it was then verified to belong to the ED cluster, and shares a majority of its characteristics with nine other ED1 subcluster phages. Its genome is 62,869 base pairs long with 119 putative genes. PhillyPhilly has a genome end characterized as a direct terminal repeat consisting of 3,380 base pairs, where genes 1-8 align with 112-119, respectively. PhillyPhilly has the ability to lyse the bacterial host and lacks an integrase gene, which led to the conclusion that it follows the lytic life cycle. Expanding upon gene functions it was also discovered that PhillyPhilly contains a RuvC-like resolvase, which is key in genetic recombination. This could gene could be essential in understanding how these phages change and evolve. Interestingly, PhillyPhilly acquired a set of three genes that are common among all ED2 cluster phages, but are only found in half of the ED1 cluster phages. These findings have helped us better understand what our phage is and how it interacts within its environment.

# 11th Annual SEA Symposium Abstract

University of South Florida

Tampa FL

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**Louis Otero**

## ***Relationships of Genome Length, Capsid Size, Tape Measure Protein, Tail Length and Functional Gene Calls Across Multiple Phage Clusters***

Alexandra Rodier, **Louis Otero**

Genome sizes in actinobactriophages range from 14,270bp to 235,841bp. Actinobactriophages also have three different morphologies: siphoviridae, myoviridae, and podoviridae, all typically characterized by tail size. This research investigated several relationships: 1) genome size to capsid size; 2) tape measure protein size compared to tail size; 3) genome size to function gene calls; and 4) genome size compared to functional gene calls with structural proteins. Five clusters were utilized that varied in genome size: Cluster C (C1) (154,830 bp), Cluster J (110,185 bp), Cluster E (75,874 bp), Cluster AK (43,397 bp), and Cluster AN (15,547 bp), and 10 phages from each were evaluated. EM images were used to measure the diameter and volumes of the capsid and determine the length of the tail. The genes list was evaluated for function and classified as structural proteins, enzymes, or regulatory proteins. T tests were used to determine statistical significance between the different measurements and regression analysis used to determine correlations. The results show that there is a significant correlation between capsid size and genome volume/length. From the 50 phages evaluated, the phage, Dandelion from Cluster C (C1) has the largest genome with a capsid size of 100 nm compared to 25 nm for the phage, Toulouse (AN) with the smallest genome. The length of the measure protein also correlates to tail size. The phage, BAKA, found in Cluster J has the longest tape measure protein with a tail size of 240 nm, while Hunnie is one of the phages with the shortest tape measure protein with a tail size of 71 nm. When relating genome size to the percentage of called functions, there is only a relationship between certain clusters. Clusters C (C1), J, and E have an average percentage of called functions of 21%, 24%, and 22%, even though the genome sizes range from 75kbp to 154kbp. However, the percent of called genes is statistically significant when these clusters are compared to Cluster AK and Cluster AN due to an average percentage of called functions of 42% for Cluster AK and 61% for Cluster AN. The data also show that as the genome size increases, the percentage of called-functional genes with structural proteins decreases. For Cluster AN, the average percentage of called-functional genes with structural proteins is at 75% compared to about 35% in Cluster C (C1). This research supports an evolutionary relationship between genome size and the size of the capsid. Future research might explore whether there are also differences in the size of capsid proteins and how the capsid is assembled. The data show that the most compact genomes have a high number of called genes of

which the majority encode structural proteins. This raises the question regarding why a phage would evolve with a genome that can be up to 10—times larger and what functions exist for the high percent of uncalled genes in one of these phage.



# 11th Annual SEA Symposium Abstract

University of South Florida

Tampa FL

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**Richard S Pollenz**

## ***Big Things in Small Packages: EK2 Cluster Actinobacteriophage Akoni and Analysis of its “Super Gene”***

Alexis Bailey, Richard S Pollenz

Analysis of phage from every cluster show that the longest genes are typically tapemeasure and very few encode proteins >2000aa. Akoni is a cluster EK2 podoviridae actinobacteriophage that has a 54,307bp genome containing 55 genes. Gene #31 is the first gene in the forward direction and it is a 13,464bp ORF (nearly 25% of its genome) coding for a protein of 4487aa making it the largest gene ever found in a phage. There are 5 EK1, 2 EM and one other EK2 phage that are also podoviridae and contain a >13,000bp gene in the same location. The goals of this project were to 1) search the bioinformatics databases to determine if other phage expressed a similar protein, and 2) determine if the protein product of gene #31 could be detected within a purified population of Akoni. A standard NCBI BLASTp analysis did not detect any significant hits, however, using PSI-BLAST (Position-Specific Iterative Basic Local Alignment Search Tool), revealed low identity hits to virion RNA polymerase (vRNAP) from *E. coli* phage Pollock and other N4-like phage. Analysis showed that 1) all of the phage encoding the vRNAP were classified as podoviridae, 2) the vRNAP gene was the first gene in the reverse or forward position, 3) the vRNAP gene encoded a very large protein of ~3,500 amino acids and 4) the vRNAP proteins did not contain cystine. Although compelling, 3D modeling, HHPred analysis and secondary structure predictions with different regions of gene #31 protein and vRNAP did not reveal any significant similarities between the two sequences. vRNAP is predicted to be associated with the capsid and responsible for early gene transcription. To determine if the gene #31 protein could be detected in Akoni, 20-25ml of high titer Akoni lysate was centrifuged, concentrated in <300ul, subject to several rounds of sonication to disrupt the phage and processed for SDS-PAGE or digested with trypsin for proteomic analysis using GC/MS. Proteomic results show that the gene #31 product was detected with high intensity spanning 52 different peptides. The coverage of gene #31 ranged from amino acid 62 to 4303 suggesting that the entire protein was translated. The products of genes #32-37 were also detected as were #40-46. The majority of genes in this region did not have many functional calls, but were predicted to be structural and included the portal protein (#32) and several minor tail proteins (#35-37). SDS-PAGE results of coomassie stained gels have not yet shown a high molecular mass protein that migrates above the 250kDa molecular mass marker but do show an intense band that migrates at ~60kDa. Gene #33 encodes a 555aa protein (60kDa) and it is possible this may be a capsid protein. These studies validate that the gene #31 product is a component of the intact phage particle. Future research will be important to determine the precise function of the protein. It is intriguing to speculate that this unique protein may be an actinobacteriophage version of the vRNAP.

# 11th Annual SEA Symposium Abstract

University of Southern Mississippi  
Hattiesburg MS

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Savannah Underwood

## ***Functional analysis of conserved hypothetical genes in the cluster K bacteriophage Hammy***

**Savannah Underwood**, Amber Coats, Sara Dao, Grace Dittmar, J.C. Gardner, Taylor Gore, Taiya Jarva, Kathleen Johnson, Giorgi Kenkebashvili, Sudiksha Kumar, Jasmine Ransom, Gabriella Reyes, Chazmyn Riley, Daniel Sinclair, Breanna Smith, Audra Thompson, Garrett Watts, Victoria Williams, Clint Pablo, Danielle Heller\*, Viknesh Sivanathan\*, Dmitri Mavrodi

\* Howard Hughes Medical Institute, Chevy Chase MD

The genus *Mycobacterium* encompasses diverse saprophytic and commensal species, as well as serious pathogens such as *M. tuberculosis* and *M. leprae*. Bacteriophages play a crucial role in the evolution of mycobacteria and provide insights into the genetics and physiology of this economically important group of organisms. Mycobacteriophages also attract a lot of recent interest as potential therapeutic agents for the treatment of multidrug-resistant tuberculosis. To date, over 1,700 bacteriophages that infect *Mycobacterium* were characterized through genome sequencing and grouped into 29 clusters based on genetic similarity. In addition to well-characterized genes that encode structural, regulatory, DNA metabolism, and lytic proteins, all mycobacteriophages genomes carry numerous conserved hypothetical genes. The specific functions of these genes remain unknown, and only a few proteins have been expressed and studied experimentally.

In this study, we performed a functional analysis of Hammy, a K cluster mycobacteriophage with a 62-kb genome that encodes 95 predicted protein-coding genes. Fifty of these genes are homologous to viral proteins of known function, while the role of the remaining 45 genes is currently unknown. We employed a combination of high-fidelity PCR and Gibson assembly to clone 81 Hammy gene into the broad-host-range plasmid vector pSMEG-ExT (85% overall success rate). The resultant recombinant plasmids were electroporated into *M. smegmatis* mc<sup>2</sup> 155 and screened for cytotoxicity in the presence of the inducer anhydrotetracycline. The screen identified several cytotoxic genes, which are variably present in members of the K cluster (immediate relatives of Hammy) and other phages that infect *Mycobacterium* and *Gordonia*. Four cytotoxic genes (32, 34, 50, and 56) were subjected to bacterial two-hybrid analysis to identify the host proteins targeted by Hammy. The two-hybrid analysis identified several *M. smegmatis* proteins targeted by the

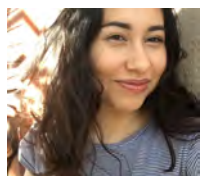
cytotoxic gene 56. One of these targets, malate synthase, was previously identified during the two-hybrid analysis of bacteriophage  $\phi$ KMV and *Pseudomonas aeruginosa*. Results of this study will help to elucidate the role of poorly characterized viral genes in the biology of phages that infect *M. smegmatis*, *M. tuberculosis*, and closely related bacteria.

# 11th Annual SEA Symposium Abstract

University of Texas at El Paso

El Paso TX

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**Lizbeth Carmona**

## ***Something with BossLady***

**Lizbeth Carmona**, Joathan Bonglo, Evelyn Cohen, Devin Moreno, Monica Ponce, Dante Reyna, Alheli Romero, Andres Vasquez, Stephen Aley, Germán Rosas-Acosta

The surge of antibiotic-resistant pathogenic bacteria has driven the search for alternative approaches to treat bacterial infections and overcome this urgent healthcare challenge. Actinobacteria phages are promising candidates for this role, given their ability to kill important human bacterial pathogens while being harmless to humans. To contribute to this research effort, we attempted the isolation of novel *Arthrobacter* phages from our local environment. However, after using the enrichment method with 24 different samples, we failed to isolate new *Arthrobacter* sp. phages from the El Paso region, probably due to the particularly dry conditions of the 2018 fall in the Chihuahuan desert. Thus, for the genomic annotation component of the course, our UTEP's Phage Hunter team was assigned the phage BossLady, isolated from Germantown, MD., by the Howard Hughes Medical Institute. BossLady has a 51,178 bp-long circular genome coding for 79 genes, 72 in the forward direction and 7 in the reverse direction. One of the reverse-oriented genes is located in an isolated position near the 5' end, a trait shared by most other members of the AO2 subcluster. Our annotation of BossLady's genomic sequence revealed an important discrepancy with the phage morphology data in the PhagesDB database. This led us to re-examine the morphotypes and approved functions assigned to some of the shared genes across members of the AO2 subcluster. At the morphological level, we found that BossLady and AO2 subcluster member BarretLemon are morphologically classified Siphoviridae even though their genomes contain genes coding for Tail Sheet and Tail Tube proteins, which are exclusive of phages displaying Myoviridae morphology. Considering that BossLady and BarretLemon exhibit close genomic association to all members of the AO2 subcluster, that all AO2 phages for which electron microscopy images are available exhibit Myoviridae morphology, and that no electron microscopy data is available for BossLady and BarretLemon, we propose that they should be reclassified as Myoviridae. At the genomic annotation level, we found that gene product 12 (gp12) showed extensive sequence identity with similarly positioned genes of most AO2 subcluster phages and that such genes had been functionally identified as head-to-tail connector proteins. However, HHPRED analyses showed that BossLady's gp12, just like all other BLAST-matching genes analyzed, lacked the required sequence relatedness with SSP1 gp15 or SSP1 gp16, which would justify their functional assignment as head-to-tail adaptor or head-to-tail stopper respectively. Thus, we did not assign the head-to-tail connector function to gp12 and propose that such function should be eliminated for similarly located genes of members of the AO2 subcluster. Our analyses indicate that, as our knowledge of the functional properties of phage-encoded genes increases, substantial refinement of previous genomic annotations will be needed.

# 11th Annual SEA Symposium Abstract

University of West Alabama

Livingston AL

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**Anna E Morse**

## ***Grabbing A1 phages by the tail: Characterization of amino acid variation and tertiary structure of the tape measure protein***

**Anna E Morse**, Brianna E Forrest, Garren R Granec, Emma D Ryan, Kayla M Fast, Tracy W Keener, Michael W Sandel

Bacteriophages were isolated from soil samples using the bacterial host *Mycobacterium smegmatis* mc<sup>2</sup>155. Instead of using a restriction enzyme digest to characterize phages before selecting which genomes to sequence, we applied subcluster-specific PCR primers targeting the tape measure protein (TMP). By comparing the sequenced products we determined whether phages matching to subclusters previously discovered at the University of West Alabama (UWA) were novel or replicated. The F2 subcluster is highly conserved across the TMP segment, and we could not determine whether our four F2 phages were unique or identical. Conversely, TMP segments from other subclusters were informative and indicated that multiple, unique phages from the same subcluster have been isolated at UWA. Our PCR results suggested that Sumter was an A1 phage. We chose to sequence this genome because UWA had not annotated an A1 phage genome before. The second phage, Candle, was selected because a cluster was not conclusively identified by PCR. The genomes of Sumter and Candle are 52,656 bp and 71,390 bp long respectively. Annotation in PECAAN and DNA Master identified 90 protein coding genes in Sumter and 96 in Candle. Next, we looked for patterns in sequence variation within the A1 subcluster. To do so, we determined synonymous codon usage bias using CAICAL and visualized protein sequence conservation in WebLogo for the following proteins: major tail protein, TMP, minor tail protein, DNA primase, helix-turn-helix DNA binding domain, membrane protein, and immunity repressor. Conservation of amino acids across members of the A1 subcluster was apparent in segments of the major tail and minor tail proteins. The other proteins investigated showed higher amino acid variability. We predicted protein tertiary structure for the seven genes listed above by using I-TASSER to match our amino acid sequences to structures in Protein Data Bank (PDB). All phages found by UWA students were visualized using a transmission electron microscope including members of the *Siphoviridae* and *Myoviridae*. Looking collectively at the sequenced phages from UWA, we confirmed the previously identified correlation between phage tail length and TMP length.

# 11th Annual SEA Symposium Abstract

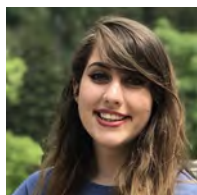
University of West Florida

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



**Cassandra Kelso**



**Naomi Semaan**

## ***A tale of DOGEMS – lessons learned out of M. foliorum phage hunting***

**Cassandra Kelso**, Andrew Brown, Katelyn Cleveland, Riley Dibble, Patrick Luciani, Jamie Martinez, Sydney Moore, Gabrielle Norman, Cassidy Paige, Mallory Vining, Werley Ryan, **Naomi Semaan**, Karen Barnes, Kari Clifton, Hui-Min Chung

This presentation is to discuss the strategies and success of using *Microbacterium foliorum* as the bacterial host for phage hunting and how we found all phages their belonged clusters. DOGEMS (DAH--jums) stands for Deconvolution of Genomes after En Masse Sequencing. It is a method used in the SEA-PHAGES community to identify clusters for a pool of phages of interest. It involves two parts: 1) En Masse Sequencing (sequencing a convoluted mixture of phage DNAs) and 2) Deconvolution of Genomes (using one or several experimental approaches to de-convolute by matching the contig DNA sequences obtained from part 1 to their respective input phages. The experimental design for part 2 in general includes: a) blasting all the contigs to identify phage clusters of interest, b) design cluster-specific primers of PCR experiments to test all the phages of interest. Since 2017 we have used the DOGEMS method to identify clusters for the phages that were not chosen for phage genome sequencing. Depending on the lengths of contigs and primer specificities, our success rates varies from 50% to 100%. Using *M. foliorum* as the host, we employed direct isolation method (37% successful rate) and enriched isolation method (7% successful rate) for phage hunting. This year using *M. foliorum* as the host, we isolated six phages by employing both direct isolation method (37% successful rate) and enriched isolation method (7% successful rate). We sequenced phages Sharkboy (in cluster EB) and Araxxi (in cluster EM), and were able to ID the rest four with the DOGEMS approach: phages BKfootlettuce and FreshAvocado in cluster EB, and phages Zepp and Alakazam in cluster EA5, both now have their phage genome drafts in the Actinobacteriophage database and Phamerator. A few interesting points emerged out of characterizing these phage genomes: 1) We found BKfootlettuce, FreshAvocado and Sharkboy derived from

the same phage ancestor, with less than 1% differences in their genomes. 2) Although Zepp and Alakazam were isolated from the soil samples of the same campus, Alakazam shares more similarity with another EA5 phage discovered in North Carolina, Neferthena, than with Zepp. 3) The Araxxi genome contains a super big gene (gp27) that encodes a protein of approximately more than 4,000 amino acid long. Interestingly, not only the orthologs of this gene were found in EM phage Burro and a few EK1 or EK2 phages like ArMaWen, TinyTimothy, and Akoni, but also partially in a hypothetical gene from Pseudomonadales bacterium. We will discuss our DOGEMS experimental design and the phage genome characterization in more detail.

# 11th Annual SEA Symposium Abstract

University of Wisconsin-River Falls

River Falls WI

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



**Kaitlyn L Fields**



**Shawna M Larson**

## ***Comparative genomics of phages isolated on new host species reveals novel genome features***

**Kaitlyn L Fields**, Ahmed Abdullah, Joe Alexander, Chanel K Cuneo, David C Ennest, Danielle Ertsgaard, Sarah B Fields, Kaylee J Fritz, Helena Humphreys, Brady T Johnson, Jacob E Kinneman, **Shawna M Larson**, Merit N Lemunyete, Moya B Murray, Noah D Nelson, Ezekiel K Olakunle, Delany E Osmond, Kelly A Patras, Sunny Ransibrahmanakul, Amy C Reimer, Cally C Robertson, Grace V Ross, Katie A Simpson, Bricarah S Thull, Sam Wetzel, J A Bonilla, Karen K Klyczek

UWRF phage hunters explored the relationships between phages isolated in different host species. We used *Microbacterium foliorum* NRRL B-24224 SEA and *Microbacterium paraoxydans* NRRL B-14843, and also *Arthrobacter globiformis* B-2979. Each soil sample was tested on all three hosts. From 53 samples, we isolated 49 phages – 13 on *M. foliorum*, 23 on *M. paraoxydans*, and 13 on *A. globiformis*. The genomes of 5 phages were sequenced: WaterT, LeeroyJenkins, and Tyrumbra from *M. paraoxydans*, and Vibaki and Qui from *A. globiformis*. We identified an additional three phages genomes so far in a DOGEMS sample, including Hubbs (*M. foliorum*), RubyRalph (*M. paraoxydans*), and Shoya (*A. globiformis*). We observed a variety of relationships between these phages and others isolated on various species of *Microbacterium* and *Arthrobacter*. Tyrumbra, Hubbs, and RubyRalph have sufficient nucleotide similarity with other phages to be assigned to clusters. Tyrumbra is in cluster EC, Hubbs is in cluster ED1, and RubyRalph is in cluster EG. These three clusters include phages isolated on both *M. foliorum* and *M. paraoxydans*. WaterT and LeeroyJenkins had no nucleotide similarity to other phages, but together they formed new cluster GB. They share 96% nucleotide identify over



78% of their genomes, and 62.5% gene content similarity. These phage genomes are 61,090-62,439 bp long, with 1545-1807 direct terminal repeats. Their overall genome structure is similar to the cluster ED1 phages, but they share only 2 phams. The *A. globiformis* phages are all Singletons, but are related to phages in existing clusters. Shoya shares 25.5% gene content similarity with cluster FB phages and 27.5% similarity with Maja, another Singleton. Shoya is the only temperate phage that we found, and we are isolating lysogens to conduct immunity testing with other *Arthrobacter* phages. Vibaki shares 24-26% gene content similarity with the cluster AO phages, and has a similar Myoviral morphology. Qui shares about 20% gene content similarity with cluster AM and AU phages. It has a prolate head, like the AM phages, but its head is much longer, 150 nm long and 50 nm wide. Its genome is 113,655 bp, compared to 58,000-59,000 for the AM and AU phages. Qui also shares 12-20% gene content similarity with a large group of phages from different hosts, including clusters AW, BI, CC, DJ, and EL. These phages all have low G+C% content, several genes encoding membrane proteins, and many intergenic repeat sequences. There are several pairs of tandemly repeated genes in Qui's genome, not found in the other phages. In addition, many Qui genes have blastp hits to other genes in the Qui genome, with e values of  $<10^{-4}$ , suggesting a history of gene duplication and divergence. These observations suggest gene duplication as one possible mechanism for the expansion of the Qui genome relative to related phages.

# 11th Annual SEA Symposium Abstract

Virginia Commonwealth University  
Richmond VA

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



**Nasita Islam**

## ***Phinding Phages & Bacteria Phrom James River Rockpools: A comparative genome analysis***

**Nasita Islam**, Sophia Fehrmann, Daanish Fiaz, Sukhleen Kaur, Rafa Khan, Mario Melchor-Guerra, Nevin Nguyen, Allison A Johnson

Bacteriophages are undoubtedly great in number; the estimated 1031 members of the phage community are biologically unique yet genomic characteristics among diverse phages may be conserved. In addition to their fascinating diversity and features, their applications in phage therapy make them an important topic of study. Analyzing phages, such as in our research project, allows for a greater understanding of phages as a contribution to the ever-growing knowledge of their impact on our biosphere as well as potential use in phage therapy.

Isolation of novel bacterial hosts from James River rockpools and using those hosts for phage discovery was piloted during Summer 2018. Students in the 2018-19 VCU Phage Lab collected water from rockpools in the James River in order to find phages and bacteria. Bacteria were isolated from the water in the rockpools by streaking a plate, and restreaking until apparent homogeneity of colony appearance. Those novel isolates were then used for phage discovery. Bacteriophages were isolated from river water through enrichment isolation and purified by plaque assays. Multiple rounds of purification were conducted to ensure consistent plaque morphology. A high titer lysate was then collected by flooding a webbed plate with phage buffer and filtering. Phage particles were placed on electron microscopy grids and stained for visualization under a transmission electron microscope. DNA was purified from lysate, and characterized by restriction digest enzymes. Two phages, named Phynn and Kyle lysed our novel bacterial isolate *Pantoea* sp. A third phage, Beyonphe, lysed a novel bacterial isolate *Bacillus cereus*.

The genomic DNA of these phages was sequenced. Following sequencing, students collaboratively annotated the genomes. Bioinformatics tools, including DNA Master, HHpred, and Blastp along with reference to Genemark coding predictions were used in order to analyze the genomes. While *Pantoea* phage Phynn, a myoviridae, had a 173,720 base pair genome with circularly permuted ends, Kyle had a genome length of 73,168 base pairs with 3,603 base pair long terminal repeat. The *Bacillus* phage Beyonphe had a genome length of 163450 bp and a 2154 bp long terminal repeat. Phynn had 263 open reading frames with two different tRNAs as well as a 44.56% GC content, while Kyle had 109 open reading frames and zero tRNAs. Phynn and Kyle have no significant similarity to each other, and little similarity to other sequenced phages in

Genbank. In contrast, Beyonphe had 292 open reading frames and no tRNAs, and significant conservation of genome sequence and proteins to previously sequenced and annotated Bacillus-infecting phages. The relative conservation of Bacillus phage genome sequences and proteins will be contrasted with the novelty of Pantoea phage genome sequences and proteins through analysis using Bioinformatics tools.

# 11th Annual SEA Symposium Abstract

Virginia State University

Petersburg VA

Corresponding Faculty Member: Andrea Beyer (abeyer@vsu.edu)



Sarah M Peebles

## ***Discovery and Comparison of E Cluster Phages Infecting *Microbacterium foliorum****

Sarah M Peebles, Kathryn H Shows, Brian L Sayre, Andrea R Beyer

Bacteriophage are the most abundant microbes on earth, at an estimated population of  $10^{31}$  particles. However, their genomes are grossly underrepresented in sequence databases, and a large portion of their genes are of unknown function. In order to gain a better understanding of phage diversity, novel viruses were isolated and characterized from soil using an Actinobacter host. *Microbacterium foliorum* is a Gram-positive, rod-shaped bacterium commonly found in soil and grass. Multiple bacteriophage were isolated from samples originating at various locations on the campus of Virginia State University and from additional sites in Virginia and North Carolina. Phage were isolated using both direct and enriched procedures, resulting in the discovery of 10 phage which were further characterized by transmission electron microscopy. Eight samples were successfully imaged; 5 were found to be the siphoviridae morphology, and 3 were podoviridae. Of these, DNA was extracted from phage TeddyBoy, Owens, and SansAfet, and submitted for sequencing. The genomes were subsequently annotated using PECAAN, DNA Master, HHPred, Phamerator, and Starterator. Though TeddyBoy and Owens were found in distinct locations in Virginia, they are both in the same phage cluster (EE), share similar genome sizes, and have a significant amount of overall sequence homology and shared synteny to one another. They also bore a strong resemblance to EE draft phage BurtonThePup, isolated from Maryland. Interestingly, TeddyBoy and SansAfet, which were isolated from different areas on the same farm in Gloucester, VA, were found to be very distinct from one another. SansAfet was placed in cluster EB, and it possessed a much larger and more complex genome, with multiple genes of unknown function. The results of this study underline the remarkable diversity of phage within geographically similar locations, as well as the intriguing similarities of phage isolated from spatially distant sites.

# 11th Annual SEA Symposium Abstract

Virginia Tech  
Blacksburg VA

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



**Brenna V DeBellis**

## ***Discovery of a recently described mobile element in novel Mycobacteriophage Camri***

Brenna V DeBellis, Stephanie M Voshell

Mycobacteriophages are genetically diverse viruses that infect bacteria in the *Mycobacterium* genus, which includes both *Mycobacterium tuberculosis* and *M. smegmatis*. Due to the continued increase of antibiotic resistance, researchers across the globe are revisiting old practices, such as phage therapy, to treat pathogenic bacterial infections. Understanding bacteriophage genetics is crucial in the development of these phage therapy treatments. The precise functions of the genes must be known in order to select the best phages to target each strain of bacteria. The aim of the SEA-PHAGES program is to discover and characterize novel bacteriophages using basic laboratory techniques and bioinformatic analyses to determine gene functions and find novel features within each genome. The goal of this project was to annotate the genome of novel bacteriophage Camri. Camri belongs to the G1 Subcluster and contains a unique genetic element only recently discovered in a small number of mycobacteriophages. Camri's genome contains a transposable element, better known as an ultra-small Mycobacteriophage Mobile Element (MPME), in the latter section where recombination typically occurs. The MPME in Camri's genome is relatively small in length and matches the MPME1 subcategory described in related phages. At this time, not much is known as to how these MPMEs specifically affect mycobacteriophage genomes, but they have been shown to alter gene regulation and expression in other organisms.

# 11th Annual SEA Symposium Abstract

Virginia Tech  
Blacksburg VA

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



**Stephanie M Voshell**

## ***A Unique Absentee: Mycobacteriophage Benvolio's Lack of an Integrase Gene***

**Stephanie M Voshell**, Ryan R Adams, Lilly S Akbary, Michael B Andrews, Carly N Baumann, Joseph P Belamarich, Priyanka S Bhuta, Caroline V Campbell, Cyrus K Crevits, Ciera R Crockett, Brenna V DeBellis, Hannah E Dolan, Cassie L Ellis, Devon N Elsasser, Shannon L Fenwick, Rui Guo, Alexis R Jackson, Ashly Kaur, Kelly A Madison, Sarah E Kivimaki, Arianna Y Martin, Samantha A McChesney, Megan R McCreary, Kylie J McMahaill, Mary K Nicely, Jessica B Ofsa, John D Redle, Michael R Santos, Katie B Stiltner, Annmarie C Taheny, Phuong V Tran, Taylan Tunckanat, Aidan P Villavicencio, Kevin J Williams, Cale E Witmer, Eleanore H Woodruff

Mycobacteriophage Benvolio was isolated at Virginia Tech from local soil using *Mycobacterium smegmatis* as a host. Benvolio produced slightly turbid plaques at 37° C and was assumed to be a temperate phage. All evidence from characterization experiments including PCR of the tape measure protein gene, tail length, and a restriction digest suggested that Benvolio belonged to the A2 subcluster. Genome sequencing revealed that Benvolio was indeed a member of the A2 subcluster and that the phage lacks an integrase gene. The majority of A2 phages, including several of Benvolio's close relatives, have an integrase gene making them capable of utilizing the lysogenic cycle (temperate phage). Benvolio, like closely related Echild, contains ParA and ParB genes close to the location of the missing integrase gene. The ParAB genes are believed to form a putative partitioning cassette which conveys the ability to form lysogens. Lytic phages are sought after as candidates for phage therapy since they are more likely to destroy the target bacteria rapidly. Benvolio warrants further study to determine whether or not it can truly form stable lysogens without an integrase gene.

# 11th Annual SEA Symposium Abstract

Virginia Western Community College  
W. Roanoke VA

Corresponding Faculty Member: Heather Lindberg (hlindberg@viriniawestern.edu)



**Elizabeth Burrell**

## ***The Isolation of Barry and the Genome Analysis of three A cluster mycobacteriophages: Dr.FeelGood, SoilDragon and NothingSpecial.***

Heather Lindberg, **Elizabeth Burrell**

At Virginia Western Community College, over the course of the 2018- 2019 academic year, we isolated Barry, a unique bacteriophage with a prolated capsid head. Barry was isolated from soil collected in the Roanoke Valley using *Mycobacterium smegmatis* mc155 as the host bacterium. Unfortunately, we were unable to successfully separate Barry from another siphoviridae phage, meaning we were unable to submit Barry for sequencing. Instead, we annotated two A cluster phages, SoilDragon and Nothing Special. We chose to annotate these cluster A phages in order to compare them to DrFeelGood, which was annotated here at VWCC last year. SoilDragon and NothingSpecial share more similarity to each other than either share with DrFeelGood. Most of the similarity lies in the left side of the genome, which is not unexpected, given that much of the left side of the genome contains the genes needed for capsid development. Hopefully, through continued comparisons between annotated phages, we will be able to gain a deeper understanding of phage genetics and the evolutionary pressures which have shaped the genomes we see.

# 11th Annual SEA Symposium Abstract

Washington University in St. Louis

St. Louis MO

Corresponding Faculty Member: Chris Shaffer (shaffer@wustl.edu)



**Nitan Shalon**

## ***Analysis of Six Novel Bacteriophages Isolated from the St. Louis Area***

Nitan Shalon, Members of Bio 192

Students at Washington University in St. Louis isolated six novel bacteriophages from the greater St. Louis area: Zuko, Phettuccine, Issmi, Bmoc, Kardashian, and Saftant. Analysis of these phage genomes contributes to the understanding of phage ecology in St. Louis, MO and the set of phages capable of infecting *Streptomyces griseofuscus*. The bacteriophages were isolated using a plaque assay, and their morphology characterized by transmission electron microscopy. Each phage was then sequenced using shotgun sequencing and subsequently annotated. The bacteriophages were placed into clusters BI, BE1, BD2, BD1, BD3, and one was in a singleton. Kardashian's BI subcluster is still being finalized, and may belong to a new, fifth subcluster. Through a BLASTn query on PhagesDB, Zuko's 82,302 bp genome has only 0.25% of its genome align to its most similar phage (Nesbitt). Due to Zuko's dissimilarity, it was placed into a singleton cluster, 64.5% of its putative proteins assigned unknown function, and many of its expected structural proteins remain unidentified. Bmoc has a large 132,885 bp genome, appears to have a lytic lifestyle, and includes an array of 41 tRNA's and 1 tmRNA. Despite Bmoc's similarity with other BE1 cluster phages, there were only 61 identifiable proteins within its 239 genes. Saftant displays strong synteny with other BD3 cluster phages and contains an Ocr antirestriction protein, which has not been previously described in PhagesDB, that inhibits type I restriction and modification systems. Within this group, Phettuccine and Issmi are the two most similar phages, and have a 49,530 bp genome with 73 genes and a 50,643 bp genome with 79 genes respectively.



# 11th Annual SEA Symposium Abstract

Washington University in St. Louis

St. Louis MO

Corresponding Faculty Member: Chris Shaffer (shaffer@wustl.edu)



**Christopher D Shaffer**

## ***The inner workings and future directions for Starterator***

Christopher D Shaffer

Starterator is a python 2.7 program originally written in the Hatfull lab by Marissa Pacey led by Dan Russell. It is designed to characterize the evolutionary context of possible start codons among a collection of orthologous genes. By using multiple sequence alignments of genes from all members of a pham the level of conservation and position of annotated start codons is visualized and the results can be used as evidence for start codon annotations of new pham member genes. This poster will present a view of the inner workings of Starterator including the underlying algorithms, it will also discuss how the results of these algorithms are presented in the graphical and textual output. Finally, examples of possible future changes will be presented and requests for user input will be elicited.

# 11th Annual SEA Symposium Abstract

Western Carolina University  
Cullowhee NC

Corresponding Faculty Member: Maria Gainey (mdgainey@email.wcu.edu)

## ***Genetic Diversity and Surprising Host Range of Forty-Two Microbacteriophages Isolated at Western Carolina University***

Brooklynn Herold, Megan Eckardt, Jamie Wallen, Maria Gainey

Western Carolina University (WCU) has been a part of the SEA-PHAGES program for the past four years. During this time WCU students have isolated and archived 84 Actinobacteriophages. The bacterial host *Microbacterium foliorum* has been used for virus isolation for the past two years. In 2017, twenty Microbacteriophages were isolated and archived but initial discovery was slow with many students performing multiple rounds of spot tests. However, in 2018 due to an increase in calcium during enrichment over half the class isolated a Microbacteriophage after only one attempt. A total of twenty-two Microbacteriophages were isolated and archived by WCU students in 2018. The Microbacteriophages discovered in 2018 had very different characteristics than those discovered in the previous year. Three of these bacteriophages Slentz, Ciel, and FuzzBuster were selected for whole genome sequencing. All three bacteriophages belong to the family *Siphoviridae*. Surprisingly, Slentz and Ciel's genomes were only 17,445 bp long and were classified as cluster EE bacteriophages. Cluster EE bacteriophages have the smallest genomes of any cluster of Actinobacteriophages and encode little else besides structural proteins. FuzzBuster's genome was 54,844 bp long and was classified as a singleton with some similarity to cluster EI bacteriophages. Our class also performed an additional host-range experiment using *Microbacterium aerolatum*, *Microbacterium paraoxydans*, and *Microbacterium testaceum* obtained from the Hatfull laboratory. Excitingly, FuzzBuster and three other bacteriophages discovered in 2018 were able to infect *Microbacterium testaceum*. We were even more surprised to discover that two cluster EA2 bacteriophages sequenced in 2017 (Andromedas and ColaCorta) readily infected all *Microbacterium* species tested. These results and the cation dependency of Microbacteriophages demonstrating expanded host range will be discussed.

# 11th Annual SEA Symposium Abstract

Western Kentucky University

Bowling Green KY

Corresponding Faculty Member: Claire Rinehart (claire.rinehart@wku.edu)



Matthew P Johnson

## **Summary Of: *Microbacterium* Phages Clancy (EA1) & LilyLou (EK1); *Arthrobacter* Phage JEGGS (AM); *Gordonia* Phage Tangerine (DE1); and *Mycobacterium* Phages Mahavrat (F1), GroupThink (A3) & WideWale (A2).**

Matthew P Johnson, Lily J Berry, Elizabeth K Guernsey, Wren A Jenkins, Deeya D Patel, Colleen S Jackson, Katie L Alexander, Sai P Boyareddygar, Jacquelyn V Buckley, Austin B Byrn, Norman T Chan, Akenpaul S Chani, Natalie G Cooper, Amara N Danturthi, Stephanie D Espinoza, Miranda L Estes, Edwin J Fields, Kacie N Gaekle, Brittney N Hackworth, Ansley N Harmon, Brandon C Hodge, Braden M Johnson, Matthew J Jones, Kelsey M Littrell, Lorenzo M Mahoney, Shifa Maryyam, Emily A McAffe, Grace M McClurg, Adam T McMahan, Logan O Mingus, Jacob M Moore, Elizabeth A Morgan, Maunil Mullick, Lukas R Negron, William J Newton, Hasitha Ramiseti, Johnthomas P Reagor, Miriam C Richardson, Nicholas R Sabotchick, Anna T Simpson, Hannah M Suter, Eleanor D Tarter, Matthew H Tauchert, Elisha P VanZant, Adrienne E Werle, Austin K White, Hope E Williams, Ashley K Wright, Bobby L Gaffney, Amanda K Staples, Naomi S Rowland, Rodney A King, Claire A Rinehart

Phages Clancy (EA1) and LilyLou (EK1) were isolated from mud and soil samples respectively, and were then enriched with *Microbacterium foliorum* where both exhibited turbid plaques. Even though the plaques were turbid, neither phage has an identifiable integrase nor repressor gene. Clancy is a Siphoviridae morphotype with a head diameter of 43 nm and tail length of 133 nm. LilyLou is a Podoviridae morphotype with a head diameter of 47 nm and a tail length of 17 nm. Clancy has a genome length of 41,555 bp and encodes 62 genes. LilyLou has a genome length of 54,388 bp and encodes 56 genes. Clancy is related to a large number of EA1 phages but LilyLou only matches one published phage, ArMaWen, another EK1 phage. An interesting feature of LilyLou, and other EK1 phages, is the length of gene 33 (13,482 bp coding for 4493 amino acids) which has no known function. EK1 phages have few known functions, not even a capsid nor protein.

JEGGS was isolated from a soil sample and enriched on *Arthrobacter sp.* ATCC 21022 and belongs to the AM cluster. It has a prolate head 37 x 60 nm and a 213 x 13 nm Siphoviridae type tail. It has a genome length of 58,287 bp that encodes 100 genes. It is a lytic phage but exhibits turbid plaques. It is most closely related to Heisenberger and Mudcat which also have prolate heads.

Tangerine was isolated from a soil sample and enriched on *Gordonia terrae* and belongs to the DE1 cluster. It is a member of the Siphoviridae family but has a flattened head that is 61 nm wide and 51 nm high (along the axis of the tail). The tail is 233 nm in length. Tangerine is a lytic phage and shows clear plaques with hazy halos near the edge of the plaque. It has a genome length of 57,306 bp and encodes 85 genes. It is related to the phage Ashertheman.

Mahavrat was isolated from a moist soil sample and was enriched and isolated using *Mycobacterium smegmatis* mc<sup>2</sup>155 as the host. Mahavrat belongs to the F1 cluster. It has a Siphoviridae morphology with a head diameter of 48 nm and a tail length of 308 nm. Mahavrat is a temperate phage with a genome of 55,945 bp. It has fairly clear plaques at 30°C.

GroupThink was isolated from a soil sample and was enriched and isolated using *Mycobacterium smegmatis* mc<sup>2</sup>155 as the host. GroupThink belongs to the A3 cluster. It has a Siphoviridae morphology with a head diameter of 42 nm and a tail length of 118 nm. GroupThink is a temperate phage with large cloudy plaques. The genome is 50,574 bp long and codes for 86 proteins and 3 tRNAs. Except at 29 bp, GroupThink is identical to Heliosoles.

WideWale was isolated from a soil sample and was enriched and isolated using *Mycobacterium smegmatis* mc<sup>2</sup>155 as the host. WideWale belongs to the A2 cluster with a Siphoviridae morphology that has a head diameter of 38 nm and a tail length of 103 nm. The genome is 53,040 bp long and is identical to phages Equemioh13 and Updawg except at 6 and 10 bp respectively. It codes for 97 genes and 1 tRNA.

# 11th Annual SEA Symposium Abstract

Winthrop University

Rock Hill SC

Corresponding Faculty Member: Kristi Westover (westoverk@winthrop.edu)



**Bethany M Wise**

## ***Host range investigation of novel bacteriophages, Rhysand, Scamander, and MonChoix, Microbacterium foliorum bacteriophages isolated from the North Catawba River Region***

**Bethany M Wise**, Hallie V Smith, Ann M Cannon, Carlos E Escoto-Diaz, Justice T Dixon, Katelyn Driggers, Christine M Dunn, Erin Emiroglu, Amanda J Foster, Braylan Jackson, Erin E Kelly, Nate C Kidd, Ellery S McNeill, Savannah A Scott, Jared T Small, Allison T Smith, Allison M Stanek, Kristi M Westover, Victoria J Frost

This is the 3rd year Winthrop University has offered a two-semester undergraduate research course as part of the SEA-PHAGES (Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science) program sponsored by the HHMI Science Education Alliance. During the fall 2018 semester, students focused on isolating and identifying unique bacteriophages using *Microbacterium foliorum* as the bacterial host. All samples were isolated from the North Catawba River Region. Individual phages were purified to obtain identical plaque morphologies and then amplified to collect high viral titer (HVT) lysates. Phage DNA was isolated and cut using restriction enzymes in tandem with a Phage Enzyme Tool (PET). DNA gel electrophoresis was used to display the DNA fingerprints of the individual phages; their patterns were then uploaded onto the PET program to predict the cluster of a phage. Two phages with high titer lysates and unique restriction digest patterns; Rhysand and MonChoix, were sent to the University of Pittsburgh for DNA sequencing. The genome annotations were performed at Winthrop University, Rock Hill, SC. Rhysand is a member of the EE cluster and contains 25 open reading frames. Its genome is 17,453bp in length with a GC content equal to 68.7%. We identified a -1 frameshift in the tail assembly chaperone proteins which is characteristic of all other annotated members of the EE cluster. MonChoix is a member of the EA cluster, and a member of the EA1 sub-cluster. MonChoix has 63 open reading frames and is 41,670bp in length, with a GC content equal to 63.4%. Members of the EA1 sub cluster are not known to contain frameshifts in the tail assembly chaperone proteins, in contrast to other members of the EA cluster. To investigate host range, these phages as well as a previously characterized WU microbacterium phage, Scamander, were tested to see whether they have the ability to infect other bacteria from the Actinobacteria phylum. The hosts include *Microbacterium liquefaciens*, *Microbacterium paraoxydans* and *Microbacterium testaceum*. Initial spot titers showed signs of infection on alternative hosts by three of the 17 phages. To date, Scamander and Rhysand appear specific for their host and MonChoix exhibits a wider host range. Comparative analysis at the genome level and further testing phenotypically will help elucidate whether these newly discovered phages use a specific host to replicate or whether mutants exist that have the advantageous ability to infect a variety of bacterial hosts in their

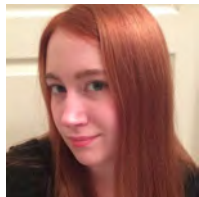
microbial community. This work adds to the increasing knowledge of bacteriophage biology and host-phage evolution, which is also relevant to the control of bacterial infectious disease.

# 11th Annual SEA Symposium Abstract

Worcester Polytechnic Institute

Worcester MA

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



**Devin G Cunningham**

## ***Comparative Genomics of EF Cluster *Microbacterium foliorum* Bacteriophages NarutoRun and Anakin***

**Devin G Cunningham**, Joseph G Crognale, Haylea T Northcott, Chloe A Sairs, Meadow R Wicke, Michael J Aquino, Vivian C Nguyen, Jennifer A Payano, Parker J Simpson, Yueqing Wang

At Worcester Polytechnic Institute, two SEA-PHAGES sponsored lab sequences were conducted, resulting in the isolation of two novel EF cluster phages: NarutoRun and Anakin. First, a bacteriophage isolation and amplification lab was conducted in Fall 2018, followed by a bioinformatics lab in which the genetic sequences of the two phages were annotated and compared to each other, other EF cluster bacteriophages, and to bacteriophages of differing clusters. The two sequenced phages were both isolated from Worcester's Institute Park, with Anakin obtained from a highly trafficked area and NarutoRun from a secluded, grassy location close to the edge of a pond.

Examining the EF cluster bacteriophages, seven genes with known functions were identified to be unique to this cluster. Of these seven two genes are tail-related, two are nondescript membrane proteins, one is the capsid maturation protease, one is an unusual DNA primase, and the last is a RuvC-like resolvase. These seven genes are present in all members of the EF cluster, but no other non-EF bacteriophages.

Upon genetic sequencing, it was discovered that both phages had circularly permuted genomes, and were identical aside from two differing nucleotides, both of which were in coding regions. The first substitution is located in a tail assembly protein, and was ultimately synonymous. The second substitution is located in a RuvC-like resolvase, and was nonsynonymous. This RuvC-like resolvase was of particular interest due to its exclusivity to the EF cluster of bacteriophages, and thus its role in regards to the management of these phages' circular genomes was investigated. With only a single nonsynonymous difference between the NarutoRun and Anakin bacteriophages, evidence therefore supports the claim that they are functionally identical phages.

# 11th Annual SEA Symposium Abstract

Xavier University of Louisiana

New Orleans LA

Corresponding Faculty Member: Joe Ross (jross@xula.edu)



**Majesty A Mason**

## ***Fun with Donkeykong: more phaging on (or at least near...) the bayou***

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Phages were isolated from a variety of locales in and around the Greater New Orleans, Louisiana area using standard microbiological techniques. Genomic DNAs from two phages were sequenced and one genome, that of Donkeykong, was chosen for analysis. The sequenced genome is 59,478bp in length, with cohesive ends showing a ten base pair overlap. BLASTn analysis reveals considerable nucleotide homology with the genomes of other known mycobacteriophages in cluster F, subcluster F1. Automated annotation employing Glimmer, GeneMark and Aragorn in the DNAMaster environment calls about 106 features, all presumptively encoding protein; no tRNA genes are predicted. Presumed lack of tRNAs and tmRNAs is confirmed by analyses run with both “external” Aragorn (i.e. via the World Wide Web external to the DNAMaster environment) and tRNAScanSE. With the help of BLASTp analysis and similar tools, it is possible to make at least tentative proposals for the functions of possible gene products in the case of a significant minority of putative protein-encoding ORFs. The structure of the genome appears to be similar to that of many mycobacteriophages, with ORFs that at least potentially encode structural products at the left end, while the right end has a more complex and difficult to predict functional picture.