Title: A Transducing Bacteriophage Infecting *Staphylococcus epidermidis* Contributes to the Expansion of a Novel Siphovirus Genus and Implies Genus is Inappropriate for Phage Therapy

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ABSTRACT

The effort to discover novel phages infecting *Staphylococcus epidermidis* contributes to both the development of phage therapy and the expansion of genome-based phage phylogeny. Here, we report the genome of an *S. epidermidis*–infecting phage Lacachita and compare its genome with five other phages with high sequence identity. These phages represent a novel siphovirus genus, which was recently reported in the literature. The published member of this group was favorably evaluated as a phage therapeutic agent, but Lacachita is capable of transducing antibiotic resistance and conferring phage resistance to transduced cells. Members of this genus may be maintained within their host as extrachromosomal plasmid prophages, through stable lysogeny or pseudolysogeny. Therefore, we conclude that Lacachita may be temperate and members of this novel genus are not suitable for phage therapy.

IMPORTANCE

This project describes the discovery of a culturable bacteriophage infecting *Staphylococcus epidermidis* that is a member of a rapidly growing novel siphovirus genus. A member of this genus was recently characterized and proposed for phage therapy, as there are few phages currently available to treat *S. epidermidis* infections. Our data contradict this, as we show Lacachita is capable of moving DNA from one bacterium to another, and it may be capable of maintaining itself in a plasmid-like state in infected cells. These phages' putative plasmid-like extrachromosomal state appears to be due to a simplified maintenance mechanism found in true plasmids of *Staphylococcus* and related hosts. We suggest Lacachita and other identified members of this novel genus are not suitable for phage therapy.

INTRODUCTION

Staphylococcus epidermidis is a Gram-positive commensal bacterium of humans that is also an opportunistic pathogen: the most common source of nosocomial infections (1). *S. epidermidis* infections are becoming increasingly difficult to treat due to the prevalence of antibiotic resistant strains (2). Instead of relying on continued development of new antibiotics (3), a promising alternative that is being approached with renewed interest is phage therapy, which uses bacteriophages to treat bacterial infections (4). Phage therapy utilizes the mechanism of lytic phage replication to kill infection-causing bacteria. While phages can be modified or selected in laboratory conditions to optimize their

performance, phage therapy relies on the diversity of naturally occurring phages of pathogenic bacteria. New phages must be isolated from the environment, characterized, and assessed for therapeutic potential (5). However, phages that infect *S. epidermidis* remain largely under-sampled and under-studied, especially in comparison to its relative, *Staphylococcus aureus* (6).

In recent years, there has been increasing research and effort to isolate *S. epidermidis* phages (7-12). As part of this *S. epidermidis* phage prospecting, members of a novel genus were isolated in multiple parts of the world in 2021. Fanaei Pirlar et al (13) isolated, characterized and sequenced a dsDNA siphovirus, CUB-EPI_14 (ON325435.2), that is ~43kb and has a narrow host range within *S. epidermidis*. Despite the genome of CUB-EPI_14 being labeled as likely temperate by PhageAI, which is normally disqualifying for a potential phage for therapy (14), CUB-EPI_14 lacks an integrase and the authors suggest it could be a potential candidate for phage therapy (13).

Simultaneous with the work of Fanaei Pirlar et al, we also isolated a related phage from this novel genus by culturing wastewater on *S. epidermidis*, as did a third group, who has deposited their phage's genome in GenBank without an accompanying paper (GenBank ON550478.1). We sequenced the genome of our representative (Lacachita) and conducted additional host range and transduction assays. We found three additional representatives of this novel genus in GenBank (including one from an *S. epidermidis* shotgun sequencing project) and analyzed these with the three cultured phage genomes. We identified in all genomes a common phage resistance gene and a partitioning protein that is associated with being maintained in a plasmid state. Combined with our observation that Lacachita can transduce antibiotic resistance genes, we propose that members of this novel genus are likely temperate and therefore inappropriate for phage therapy.

MATERIALS AND METHODS

Wastewater Sample Screening

Aliquots of wastewater influent from a treatment plant in the mid-Atlantic United States were obtained in March 2021 and were screened for lytic phages effective against *S. epidermidis* 1457 (15). 5mL of the wastewater samples were combined with 0.15g powdered tryptic soy broth (TSB) medium, 25µL of 1M $CaCl_2$, and 50µL bacterial broth culture (*S. epidermidis* 1457), then incubated overnight at 37℃. A second sample of wastewater underwent the same procedure without the addition of host bacteria. After incubation, the mixtures were centrifuged at 3220xg for 15 minutes and the supernatant was passed through a 0.22 µm filter. 100µL of the filtrate was cultured with 100µL of 10^{-1} bacterial dilution of an overnight culture using the pour plate method (in 3mL of 0.3% molten TSA combined with 25µL of 1M $CaCl_2$, vortexed and poured onto TSB 1% agar plates). The plates were then incubated overnight at 37℃ and examined for the presence of plaques.

Phage Isolation

In order to purify phages identified during the screening process, isolated plaques were picked up using a sterile glass pipette tip and the agar was deposited into a culture tube containing 2mL TSB, 50µL bacterial broth culture, and 25µL of 1M $CaCl_2$, and was incubated overnight at 37℃. This liquid culture was then centrifuged at 3220xg for 15 minutes and the supernatant was passed through a 0.22µm filter. The filtrate was diluted, and 100µL of this diluted filtered supernatant was combined with 100µL 10^{-1}

bacterial dilution, 25μL of 1M $CaCl_2$, and 3mL of molten TSA, vortexed, and poured onto tryptic soy agar (TSA) plates. The plates were then incubated overnight at 37℃ and examined for plaques. This subculturing procedure was performed a total of three times to yield a purified, enriched phage stock.

DNA Isolation and Sequencing

The DNA genome of one isolated phage was extracted using a Qiagen QIAamp MinElute Virus Spin Kit. Paired end Illumina sequencing was performed at MiGS (Microbial Genome Sequencing Center, now SeqCenter). Reads were analyzed using CPT Galaxy Phage genome assembler v2021.01 Workflow (16), which uses SPAdes Galaxy v3.12.0 (17). This yielded three assembled contigs. These contigs were aligned manually using Aliview (18) to verify that they were identical (except for short regions of duplication due to the likely circular genomes having been assembled linearly) and to produce a complete genome without such duplications. The Lacachita genome was reoriented to mimic the linearization of relatives found using NCBI Standard Nucleotide BLAST.

Genome annotation

The Lacachita genome was annotated using Prokka (v1.14.6, Galaxy; parameters Kingdom: Viruses, (19). Predicted ORFs were annotated further using NCBI Standard Protein BLAST, and the sequences producing significant alignments were analyzed to determine functional gene annotations for Lacachita. When the BLAST search produced multiple identical hits, we chose the annotation that was most relevant to a phage lifestyle (eg: the name given in another phage genome). Phylogenetic analysis of the Lacachita genome and other dsDNA phage genomes was performed with GRAViTy v1.1.0 (Genome Relationships Applied to Virus Taxonomy, http://gravity.cvr.gla.ac.uk/,(20).

Further functional annotation was performed. Promoter sequences were predicted by inputting the Lacachita genome into the Genome2D Prokaryote Promoter Prediction tool (21). Rho-independent termination sites were predicted using the ARNold web tool (22). Noncoding RNAs were found using Rfam (23). TRNAscan-SE was used to search the Lacachita genome for transfer RNAs (24).

Comparison to related phage genomes

To identify close relatives of Lacachita, the assembled Lacachita genome was used to query NCBI Standard Nucleotide BLAST. Other phage relatives were identified by searching predicted Lacachita ORFs using NCBI Standard Protein BLAST and making note of organisms with consistent protein homology to Lacachita ORFs, whose genomes were then compared to Lacachita directly using NCBI Align Sequences Nucleotide BLAST. Lacachita and identified relatives were analyzed with GRAViTy v1.1.0 to determine their taxonomy (20). GRAViTy results were visualized using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). The protein products of Lacachita predicted ORFs were analyzed to identify potential indicators of phage lifestyle, and the genomes of Lacachita and its relatives were also analyzed using the PhageAI lifestyle classifier algorithm (25).

ParB Protein Maximum Likelihood Tree

Two sets of ParB-like protein sequences were collected for phylogenetic analysis: Lacachita ParB BLASTp hits and annotated ParB sequences from *Staphylococcus* genomes. Sequences were aligned by MUSCLE (26) and the alignment was checked by eye. The aligned sequences were used to build a

maximum likelihood tree with PhyML (27) on the Montpellier Bioinformatics Platform (atgc-montpellier.fr/phyml/). The LG substitution model with empirical amino acid frequencies and estimated proportion of invariant sites were used, and 1000 bootstrap replicates were run.

Host Range

The host range of Lacachita was explored via spot plating on multiple strains of *S. epidermidis* and several other *Staphylococcus* species isolates. The *S. epidermidis* strains tested were: 1457, 158-22, B138-22, B72-22, B76-22, B64-22, NRS101 (RP62a), and ATCC 12228. We tested other *Staphylococcus* species isolates: *S. hominis* (160-22, B124-22), *S. haemolyticus* (B1869-21, 157-22), *S. simulans* (B149-22, B1781-21), *S. capitis* (B65-22, B1931-21), *S. lugdunensis* (B50-22), *S. warneri* (B21-22), and *S. aureus* (LAC WT, SH1000, MW2, N315). With exception of *S. epidermidis* strains 1457, ATCC 12228, NRS101 (RP62a) and the *S. aureus* strains, the isolates are deidentified clinical isolates that were collected at the University of Nebraska Medical Center Clinical Microbiology Laboratory. Pour plates of each strain were prepared by combining 3mL of 0.7% molten TSA, 25µL of 1M $CaCl_2$, and 10µL bacterial overnight culture, vortexing the mixture, and pouring it onto TSA 1% agar plates. Once the top agar solidified, 5µL of a dilution series (1 to 10^{-9}) of high titer Lacachita lysate was spotted onto the surface. As a control, 5µL of TSB was also spotted onto the plates. The plates were then incubated overnight at 37℃ and examined for evidence of lysis. Experiments were conducted in triplicate.

Transduction

In order to determine whether Lacachita possesses transducing abilities, a plasmid transduction experiment was performed. A modified *S. epidermidis* strain (1457 saeR/pNF155) carrying a 9kb plasmid which is marked with an erythromycin resistance gene served as a donor strain and erythromycin sensitive *S. epidermidis* 1457 served as a recipient strain (28). Phage-bacterial cocultures were prepared with 2mL of *S. epidermidis* 1457 saeR/pNF155 overnight culture (grown in TSB with 10µg/mL erythromycin) was combined with 5mL TSB, 100µL 1M $CaCl_2$, and 100µL Lacachita purified phage stock. These bacteria-phage cocultures were incubated overnight at 37℃. The following day, Lacachita phages were harvested from the cocultures by centrifugation (13,000xg for 3 minutes) and the resulting supernatant was then filtered using sterile 0.22µm filters to remove bacterial cells. This filtered supernatant was then combined with overnight cultures of *S. epidermidis* 1457 recipient strain: 500µL *S. epidermidis* 1457 culture, 500µL TSB, 100µL 1M $CaCl_2$, and 100µL of the harvested Lacachita donor phage preparation. These cocultures were incubated at 37°C for 1 hour. Following incubation, 400µL of 1M sodium citrate was added to each, each tube was vortexed to mix, and each coculture was transferred to a microcentrifuge tube. Cells were pelleted by centrifuging at 13,000xg for 2 minutes and the supernatant was discarded. The cells were resuspended in 1mL TSB and centrifuged again at 13,000xg for two minutes. The cells were then resuspended in 200µL TSB and plated on TSA plates containing 10 µg/mL erythromycin and 2mM sodium citrate. As negative controls, erythromycin sensitive *S. epidermidis* 1457 was plated on erythromycin-containing TSA plates and the Lacachita phage stock was spotted onto erythromycin-containing TSA plates. Inoculated plates were incubated overnight at 37℃ and were then examined for the presence of bacterial growth. The transduction experiments were performed six times.

Assessing Phage Resistance of Lysogenized Bacterial Cells

To determine whether transduced bacteria were resistant to new lytic Lacachita infection, we spotted serial dilutions of Lacachita lysate onto lawns of transduced *S. epidermidis* 1457 cells. We obtained transduced cells using a modification of the transduction protocol above (excluding sodium citrate from the agar plates) and representative resulting colonies were streaked onto erythromycin-containing TSA plates. Liquid cultures were inoculated with isolated colonies in 10mL TSB containing 10 μg/mL erythromycin and were incubated overnight at 37℃. Pour plates of lysogenized cells were prepared by combining 3mL of 0.7% molten TSA, 25μL of 1M $CaCl_2$, 10μL bacterial overnight culture, and 3μL 10 μg/mL erythromycin, vortexing the mixture, and pouring it onto TSA plates. Once the top agar solidified, 5μL of a dilution series (1 to 10^{-9}) of high titer Lacachita lysates were spotted onto the surface. As a control, 5μL of TSB was also spotted onto the plates. For comparison, this was also performed on *S. epidermidis* 1457 that had not undergone transduction and potential lysogeny. The plates were then incubated overnight at 37℃ and examined for evidence of lysis. Experiments were conducted in triplicate.

RESULTS

Isolation of Lacachita

Phages capable of forming plaques on *S. epidermidis* 1457 were successfully isolated from samples of wastewater influent from a treatment plant in the mid-Atlantic United States. Concentrated samples of the unenriched wastewater did produce plaques on *S. epidermidis* 1457, but the enriched wastewater produced orders of magnitude more plaques. Plating the host alone (without wastewater) did not produce any plaques. During the isolation procedure, a total of 11 plaques were harvested for potential further work. Of these isolated plaques, two were chosen for sequencing. Upon sequencing and assembly of the genomes, it was discovered that the two were 100% identical and so only one (Lacachita) was further characterized.

Lacachita genome and annotation

Lacachita has a 46,473bp dsDNA genome that is likely circular (GenBank accession OP142323). It contains 72 predicted ORFs, 19 putative promoters, 1 putative noncoding RNA (which encodes a group I catalytic intron), 19 putative rho-independent terminators, and no predicted tRNAs. Several similar phage genomes were identified by BLAST (>95% identity over ≥93% of the genome), and the Lacachita genome was linearized and oriented to mimic the genomes of its close relatives, which were also isolated on *S. epidermidis* (ON550478.1, ON325435.2, Table 1).

Lacachita's putative protein products contain an expected assortment of phage proteins and some hypothetical proteins (Figure 1). Nine structural proteins were identified, which were similar, by BLASTp, to those of siphoviruses with long, non-contractile tails. Lacachita has both a holin and an endolysin, and 14 proteins involved in DNA replication and metabolism were identified. Two ORFs are associated with a plasmid prophage lifestyle: a parB-like protein and a potential phage resistance protein. The remaining 43 ORFs in the Lacachita genome are either hypothetical (34 ORFs), are identified only with a protein family or as including a known domain (9 ORFs).

Lacachita is part of novel genus, along with other proposed members

Four other phage genomes were identified by BLASTn to be relatives of Lacachita, and another by BLASTp (Table 1). Of these five relatives, only CUB-EPI_14 (ON325435.2) has yet been thoroughly described (13). The authors of that paper noted that CUB-EPI_14 appears to represent a novel genus and identifies two other potential members of the genus via calculation of intergenomic distance: Uncultured Caudovirales phage clone 9S_3 (MF417888.1) and TPA: Myoviridae sp. isolate ct5pN1 (BK030923.1). These two phages were also independently identified as relatives of Lacachita during our searches, and so our analysis complements and bolsters the evidence for these phages representing a new genus. The genome of another cultured phage, Sazerac (ON550478.1), was deposited in GenBank after the manuscript about CUB-EPI_14 was submitted for publication, and we propose that Sazerac is also part of this novel genus. The final relative, Sep_B35_CVC_2019 (NZ_CAJUVG010000006), was identified due to its consistent protein sequence identity to Lacachita protein products. Although Sep_B35 is catalogued in NCBI as a contig of a *S. epidermidis* whole shotgun sequence, we argue that this contig represents a full phage genome from an infected *S. epidermidis* strain. Further, since the sample of *S. epidermidis* was sequenced as a bacterial shotgun sequencing project, not labeled as a study in phage infection, we suggest that the Sep_B35 genome represents a prophage that was being maintained within the *S. epidermidis* isolate at the time it was sequenced.

Taxonomic assignment of Lacachita and its relatives confirmed that these phages represent a novel genus within the family *Siphoviridae* (Figure 2). The six genomes form a monophyletic clade, clustered near the *Sextaecvirus* infecting other Staphylococci, among other siphoviruses. There was strong support for this group forming a novel genus (Symmetrical Theil's uncertainty correlation 0.863). Genomic maps of the six members of the putative genus reveal some observable regions of synteny (Figure 3).

Following the example of Fanaei Pirlar et al (13), we ran the six genomes through the PhageAI lifestyle classifier. We found that Lacachita and all members of this putative novel genus were predicted to be temperate with at least 99.95% confidence (Table 1). Three of the five proposed relatives of Lacachita are uncultured, putative phages. Based on the length and query cover of these genomes compared to those of cultured isolates, it is likely that these represent essentially complete genomes.

Host range results

Of the 7 *S. epidermidis* strains tested in this project, 5µL of Lacachita lysate was found to be capable of lysing *S. epidermidis* strains 1457, NRS101 (RP62a), B72-22, 158-22, and B138-22. Of the other *Staphylococcus* species strains tested, 5µL of Lacachita lysate was found to be capable of lysing *S. capitis* B65-22 and B1931-21 and *S. lugdunensis* B50-22 (Table 2).

Lacachita is capable of transduction and lysogeny

Transduction assays were conducted three separate times and in 5/6 cases, Lacachita was capable of transducing plasmid-encoded erythromycin resistance to erythromycin-sensitive *S. epidermidis* 1457.

Spotting of serial dilutions of Lacachita lysate on bacterial lawns of transduced *S. epidermidis* 1457 revealed that these transduced cells were 100x less susceptible to lysis compared to *S. epidermidis* 1457 that had not undergone transduction. While the highest dilution of the lysate able to produce lysis on *S.*

epidermidis 1457 was 10^{-6}, the highest dilution of the lysate able to lyse transduced *S. epidermidis* 1457 was 10^{-4}.

We were unable to identify a putative integrase gene in the genomes of Lacachita or its close relatives. ORF analysis of Lacachita and its close relatives revealed the presence of putative *parB* and common phage resistance gene (Figure 1) in all six genomes (Table 3), which is partial evidence that these phages are temperate and suggests the prophages are maintained extrachromosomally. We elected to classify Lacachita ORF70 (UVD33307.1) "ParB protein" because the highest BLASTp result (99.8% identity) was annotated as a ParB protein (MAG TPA: ParB protein [Myoviridae sp.] DAI53229.1). We conducted a phylogenetic analysis of the ParB-like proteins from this putative genus and related sequences (identified by BLASTp) and annotated ParB proteins from *Staphylococcus* genomes (Figure 4). The sequences from Lacachita's putative genus form a robust clade (99.7% bootstrap support), and several of the more distantly related sequences from other phage genomes are also classified as ParB proteins. The sister group (DAT62215.1) shares 51.2% identity and 99% query cover with Lacachita's ParB protein. While some bacterial ParB proteins were identified by the BLASTp search they were not from *Staphylococcus* – the *Staphylococcus* ParB sequences formed an outgroup to the sequences identified by BLASTp. Our decision to classify this ORF *parB* was bolstered by the efficiency of plating results on transduced *S. epidermidis* cells; Lacachita appears to be temperate. We elected to classify Lacachita ORF30 (UVD33267.1) as a "resistance protein" because its highest NCBI BLASTp result (100% identity) was the "resistance protein" DAI53234.1 MAG TPA: resistance protein [Myoviridae sp.]. The other BLASTp hits (within and outside of this putative genus) are from phage and *Staphylococcus* proteins with Siphovirus-gp157 protein family annotations (pfam05565), members of which have been experimentally shown to confer phage resistance (29, 30).

DISCUSSION

Lacachita is part of a novel genus.

Our GRAViTy analysis strongly suggests that Lacachita and its relatives belong to a siphovirus genus recently described by Fanaei Pirlar et al (13), who characterized CUB-EPI_14 as belonging to a novel genus along with 9S_3 and ct5pN1. We have also expanded this putative genus by two more phages: Sazerac and the Sep_B35_CVC_2019 putative prophage. Based on the high genetic identity between CUB-EPI_14 and the other genomes we can assume all members of this genus have long, non-contractile tails (13). Members of this genus have already been found on three continents and we anticipate further isolates will be characterized in the upcoming years. Additional hosts may be identified for these phage as well, as we have expanded the potential hosts for members of this genus to include other *Staphylococcus* species than *S. epidermidis* (relative to Fanaei Pirlar et al (13)).

Lacachita and its relatives are not suitable for phage therapy.

However, unlike Fanaei Pirlar et al (13), we do not think members of this genus should be used for phage therapy. There are several characteristics that are typically screened for when assessing whether a phage could be used for phage therapy, including: host range, phage virulence, transduction potential, stability against environmental pressures, and the presence of toxin genes (31). Bioinformatic analysis suggests members of this genus are temperate, which is contraindicated for phage therapy. Temperate phages capable of transduction have the potential to increase the pathogenicity of lysogenized bacteria

by carrying virulence factors between hosts (32). This is observed in temperate phages of *S. epidermidis* that can mobilize antibiotic resistance plasmids (33). In the interest of self-preservation, prophages also typically cause lysogenized bacteria to become immune to lytic infection by other phages that share similar repression systems (31). None of the members of this putative genus have an integrase gene, which is a key indicator of a temperate lifestyle because it allows stable integration of the phage genome into that of its host (14). Instead, the signal that PhageAI is picking up on in the phage genomes may be the presence of the *parB* gene (typically found as a *parA-parB* pair, implying a ParAB-*parS* system for chromosome segregation (34)) and the putative phage resistance gene, which is not required in phage that only rapidly lyse their host cells (35). Some temperate phages are known to be maintained in their bacterial host cells as extrachromosomal circular plasmids and maintain their presence in their hosts with similar mechanisms to plasmids (35-37). Some phage prospecting projects anticipate that these plasmid-like prophages may be isolated (38), but require that genomes have both *parA* and *parB* partitioning protein ORFs identified to be considered temperate (https://seaphages.org/forums/topic/4367/). To our knowledge, there are no characterized phage, temperate or otherwise, that only contain a gene for ParB, which binds to specific DNA sequences (*parS*, which vary among bacteria (39)). A BLASTp search with the ParB of Lacachita only found members of its genus and 50% coverage to other phage proteins (typically the N-terminus of ParB, data not shown). Nonetheless, we did not find a ParA homolog, which is an ATPase that assists with localization of ParB (40, 41) in these six phage genomes.

The hosts of these phage, which is confirmed to be *Staphylococcus epidermidis* for four of these six phages, may offer explanation. Members of families *Streptococcaceae* and *Staphylococcacae* are known to not use a ParAB-*parS* system to ensure their own chromosome's proper segregation into daughter cells; they use a ParB-*parS* system without a ParA (39). Plasmids of these hosts have been found that also use a ParBS system, such as *S. aureus* plasmid SK1 (42). Therefore, extrachromosomally maintained prophages of these hosts may also not need a ParA in order to stably vertically transmit to daughter cells. Our attempts to identify a *parS* site in Lacachita and its relatives, based on identity to *parS* sites from *S. epidermidis* (39) have been unsuccessful. However, there is low sequence identity between the ParB proteins of these phages and *S. epidermidis* and there is reason to assume that the host and phage would use quite divergent *parS* sites to bind their very divergent ParB proteins. Sequenced phages that encode both a ParA and ParB protein do not always identify a *parS* site (38) so we do not view our inability to find a *parS* site a barrier to suggesting Lacachita and its relatives are temperate phages which use a ParBS system.

There is no perfect test for whether a phage is temperate and capable of creating a lysogen. We observed intermittent turbidity of plaques on *S. epidermidis* 1457 and in the spot plating experiments on other *Staphylococcus* strains, which is often considered an important phenotype of temperate phages (35). However, turbidity can be affected by many factors (35). Importantly, we have repeated evidence of Lacachita's capacity to transduce erythromycin resistance to a previously susceptible strain of *S. epidermidis*. Transduction is a phenomenon typically associated with temperate phage, though it can be due to 'pseudolysogeny,' or the formation of a carrier state (43-45). Our experimental evidence of Lacachita's ability to confer phage resistance to transduced cells is more indicative of lysogeny. Many temperate phages do not confer complete resistance to infection with that phage and a 100-fold reduction in efficiency of plating is consistent with the behavior of other temperate phages (46, 47).

Regardless of the durability of lysogeny with Lacachita, any transducing ability is empirical evidence that Lacachita and its close relatives should not be used in phage therapy.

Phage therapy remains a promising avenue of research for treating *S. epidermidis* infections, but members of this genus are not appropriate therapeutic agents. Genetic engineering is one way to modify temperate phages to be more appropriate therapeutic candidates (48) but that approach is controversial (49). Additional isolation of *S. epidermidis* phages is needed to find obligately lytic phage.

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Figure 1: Lacachita genomic map. ORFs are annotated with predicted protein products.

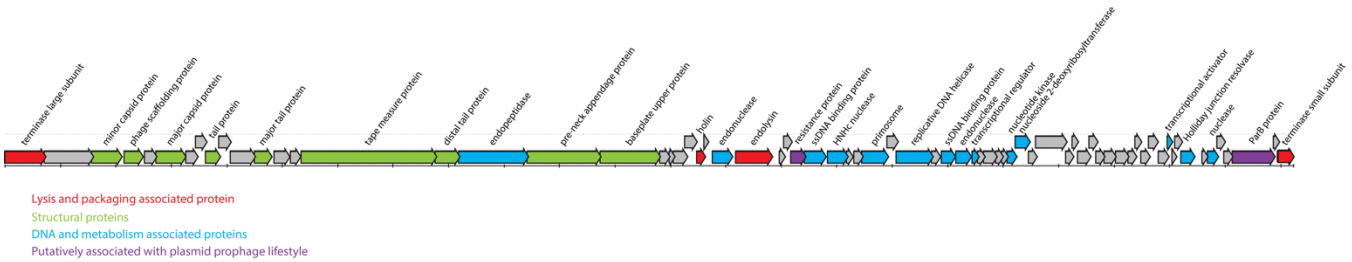


Figure 2: Phylogenetic tree of dsDNA prokaryotic viruses from GRAViTy, collapsed to focus on Lacachita and its relatives. Labels include GenBank accession numbers, family, order, and genus assignments, and phage names. The six genomes comprising the novel genus, including Lacachita, are in the blue clade.

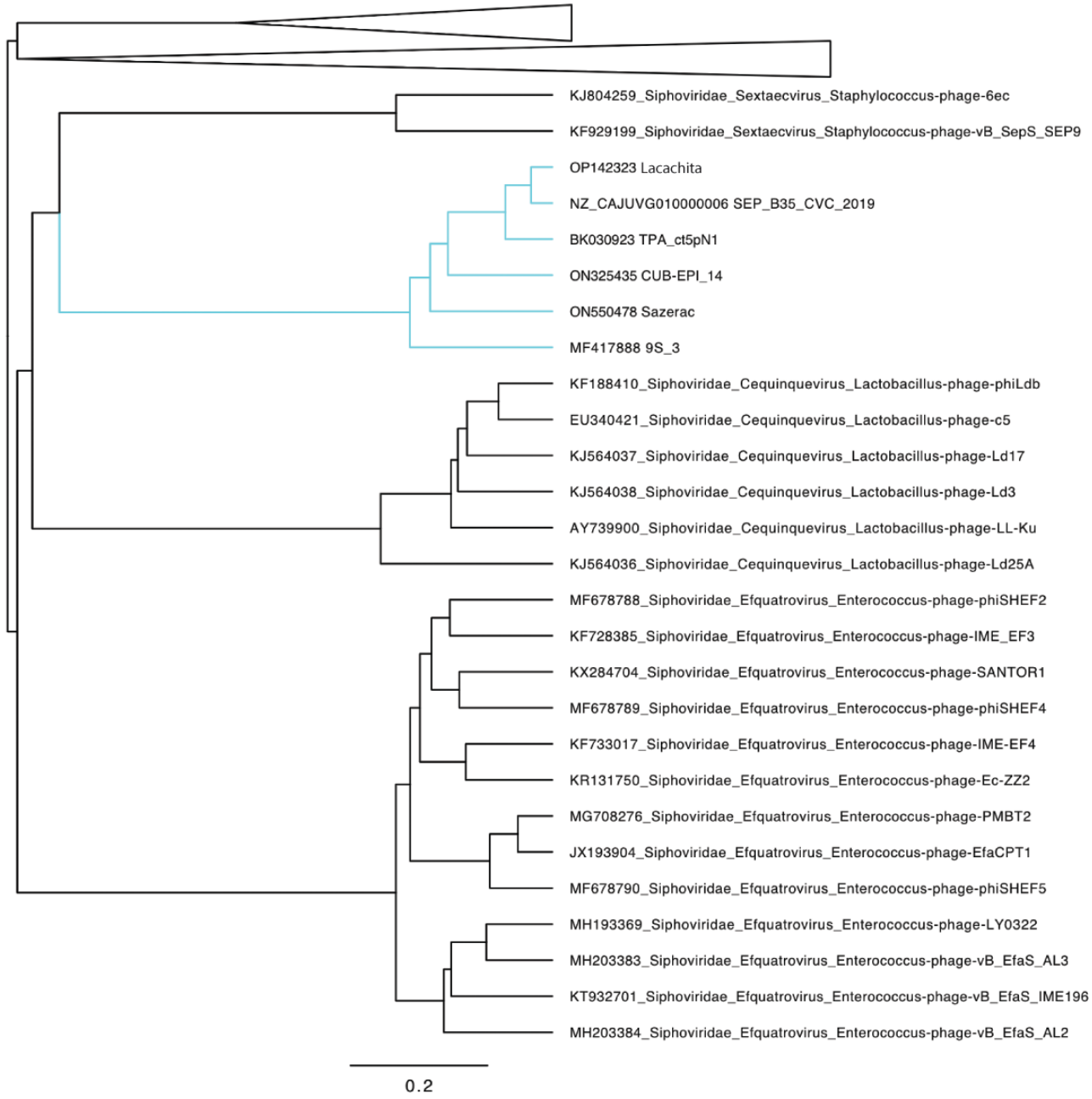


Figure 3: Genomic maps of close relatives of Lacachita. ORFs are color coded according to the categories of predicted protein products described in GenBank accessions. Genomes are presented using the coordinate numbering system from their GenBank entries. The genomes of MF417888 and NZ_CAJUVG010000006 were reversed to match the orientation of Lacachita.

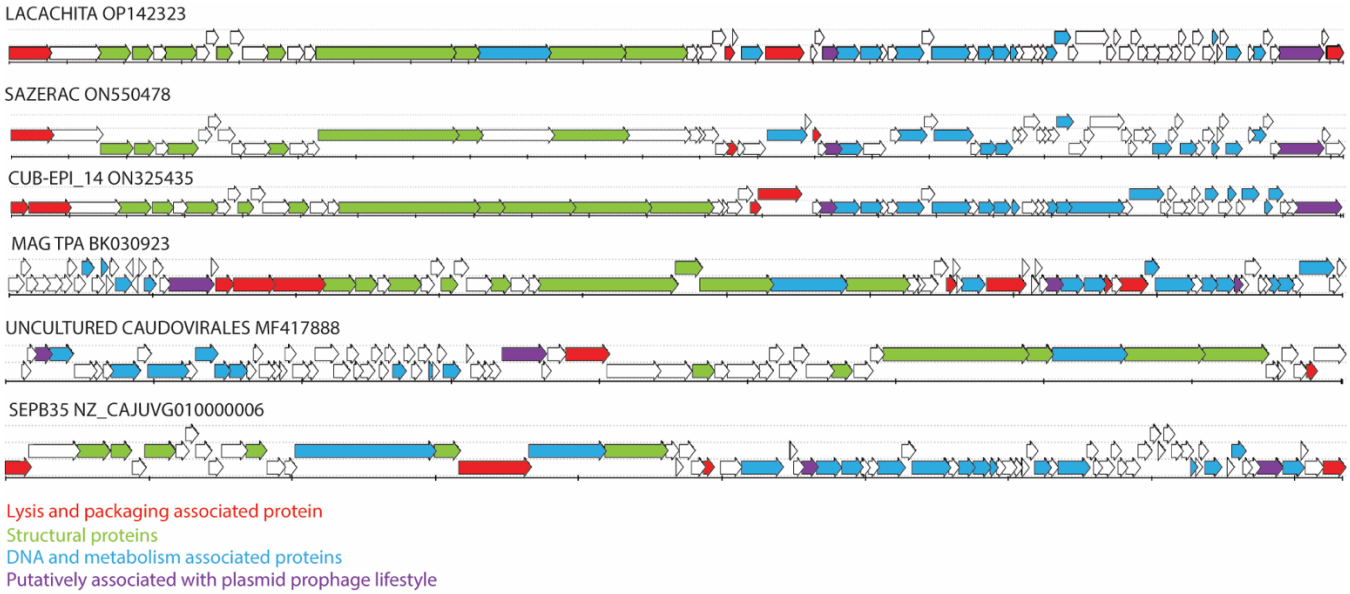
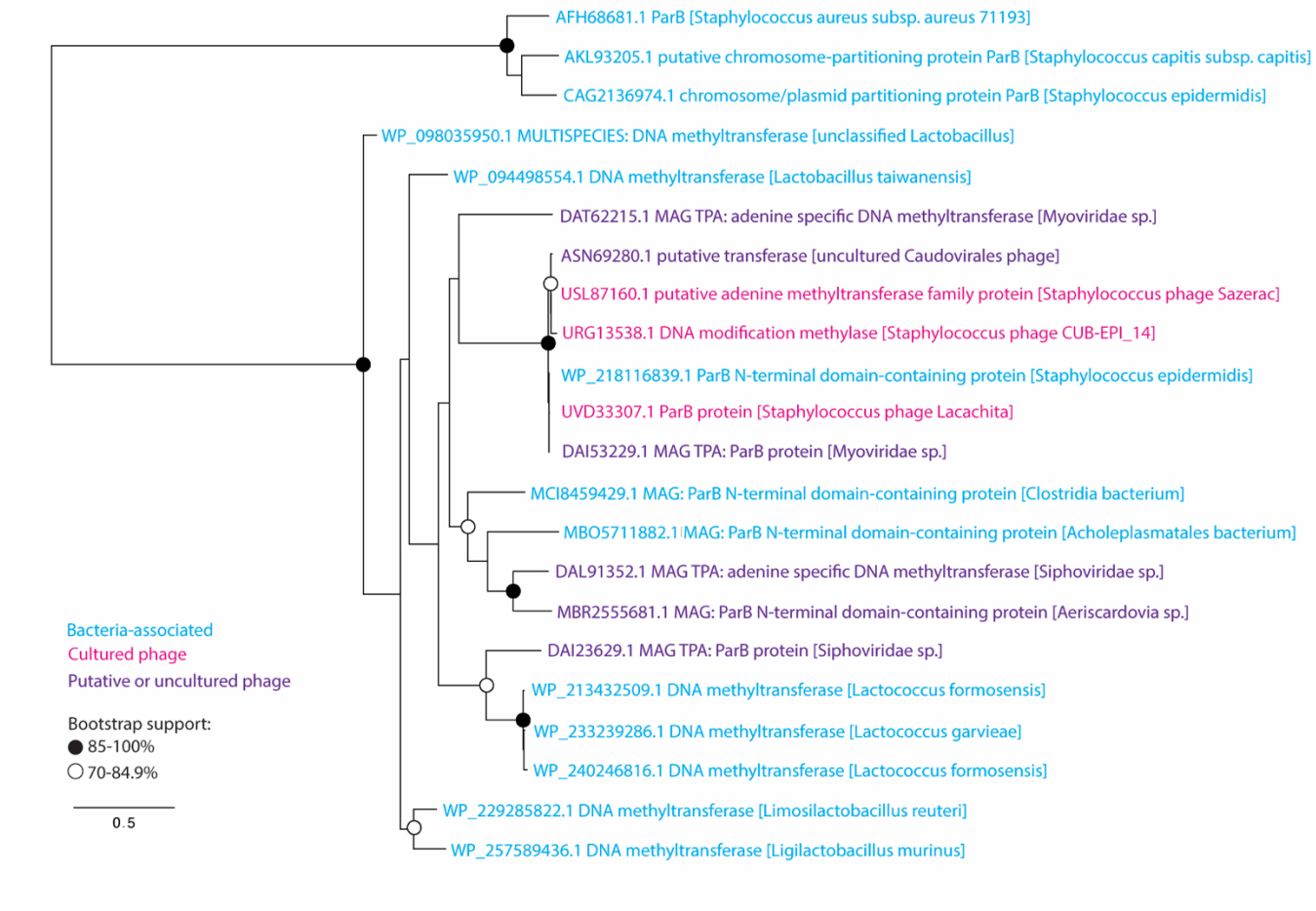


Figure 4: Maximum likelihood tree of protein sequences similar to Lacachita's ParB protein (UVD33307.1) and identified ParB proteins from Staphylococcus genomes. Protein names are color-coded according to their source organism. Bacteria-associated proteins are shown in blue, cultured phage proteins are shown in pink, and putative or uncultured phage proteins are shown in purple. Clades with moderate or strong bootstrap support are shown with open (70-84.9%) or closed (85-100%) circles.


AFH68681.1 ParB [Staphylococcus aureus subsp. aureus 71193]
AKL93205.1 putative chromosome-partitioning protein ParB [Staphylococcus capitis subsp. capitis]
CAG2136974.1 chromosome/plasmid partitioning protein ParB [Staphylococcus epidermidis]
WP_098035950.1 MULTISPECIES: DNA methyltransferase [unclassified Lactobacillus]
WP_094498554.1 DNA methyltransferase [Lactobacillus taiwanensis]
DAT62215.1 MAG TPA: adenine specific DNA methyltransferase [Myoviridae sp.]
ASN69280.1 putative transferase [uncultured Caudovirales phage]
USL87160.1 putative adenine methyltransferase family protein [Staphylococcus phage Sazerac]
URG13538.1 DNA modification methylase [Staphylococcus phage CUB-EPI_14]
WP_218116839.1 ParB N-terminal domain-containing protein [Staphylococcus epidermidis]
UVD33307.1 ParB protein [Staphylococcus phage Lacachita]
DAI53229.1 MAG TPA: ParB protein [Myoviridae sp.]
MCI8459429.1 MAG: ParB N-terminal domain-containing protein [Clostridia bacterium]
MBO5711882.1 MAG: ParB N-terminal domain-containing protein [Acholeplasmatales bacterium]
DAL91352.1 MAG TPA: adenine specific DNA methyltransferase [Siphoviridae sp.]
MBR2555681.1 MAG: ParB N-terminal domain-containing protein [Aeriscardovia sp.]
DAI23629.1 MAG TPA: ParB protein [Siphoviridae sp.]
WP_213432509.1 DNA methyltransferase [Lactococcus formosensis]
WP_233239286.1 DNA methyltransferase [Lactococcus garvieae]
WP_240246816.1 DNA methyltransferase [Lactococcus formosensis]
WP_229285822.1 DNA methyltransferase [Limosilactobacillus reuteri]
WP_257589436.1 DNA methyltransferase [Ligilactobacillus murinus]
Bacteria-associated
Cultured phage
Putative or uncultured phage
Bootstrap support:
85-100%
70-84.9%
0.5

472

473

Table 1: Other Proposed Members of Novel Genus

Phage Genome	Accession	Sample type	Isolation location	Length (bp)	Query cover of Lacachita	% identity with Lacachita	Phage AI Predicted Lifestyle
***Staphylococcus* phage Sazerac, complete genome**	ON550478.1	cultured isolate	IL, USA	46428	94%	96.27	Temperate
***Staphylococcus* phage CUB-EPI_14, complete genome**	ON325435.2	cultured isolate	Germany	46098	93%	96.19	Temperate
Uncultured Caudovirales phage clone 9S_3	MF417888.1	uncultured isolate	South Africa	45052	93%	95.53	Temperate
TPA: Myoviridae sp. isolate ct5pN1	BK030923.1	metagenome assembled genome	USA	46472	95%	98.64	Temperate
Contig of *Staphylococcus epidermidis* isolate Sep_B35_CVC_2019, whole genome shotgun sequence *(proposed prophage)*	NZ_CAJUVG010000006	whole genome shotgun sequence from *S. epidermidis* isolate	Portugal	46658	98%	96.47	Temperate

Table 2: Lacachita host range as determined by 5μL spots of serial dilutions of lysates.

Species	Strain	Highest dilution of Lacachita lysate that produced lysis
S. epidermidis	1457	10^{-5}
	158-22	lysate
	B138-22	lysate
	B72-22	lysate
	B76-22	-
	B64-22	-
	NRS101(RP62a)	10^{-2}
	ATCC12228	-
S. capitis	B65-22	10^{-2}
	B1931-21	10^{-2}
S. lungenensis	B50-22	lysate
S. haemolyticus	B1869-21	-
	157-22	-
S. hominis	160-22	-
	B124-22	-
S. simulans	B149-22	-
	B1781-21	-
S. aureus	B21-11	-
	SH1000	-
	MW2	-
	N315	-
S. warneri	B21-22	-

480 Table 3: Protein Comparison

Phage Genome	Original annotation	Accession	Query cover compared to Lacachita	% identity with Lacachita
ParB Comparison				
Sazerac	putative adenine methyltransferase family protein	USL87160.1	100%	98.20%
CUB-EPI_14	DNA modification methylase	URG13538.1	100%	95.99%
Uncultured Caudovirales phage clone 9S_3	putative transferase	ASN69280.1	90%	97.79%
TPA: Myoviridae sp. isolate ct5pN1	ParB protein	DAI53229.1	100%	99.80%
Sep_B35_CVC_2019 proposed prophage	ParB N-terminal domain-containing protein	WP_218116839.1	52%	100%
Phage Resistance Protein Comparison				
Sazerac	hypothetical protein	USL87122.1	100%	96.89%
CUB-EPI_14	hypothetical protein	URG13503.1	100%	83.23%
Uncultured Caudovirales phage clone 9S_3	hypothetical protein	ASN69320.1	100%	88.82%
TPA: Myoviridae sp. isolate ct5pN1	resistance protein	DAI53234.1	100%	100%
Sep_B35_CVC_2019 proposed prophage	siphovirus Gp157 family protein	WP_218116871.1	100%	83.23%

481

482