PHAGE TAXONOMIC CLASSIFICATION: CHALLENGES, CURRENT TOOLS, AND LIMITATIONS

Yilin Zhu  
Dept. of Electrical Engineering  
City University of Hong Kong  
Kowloon, Hong Kong SAR, China  
zy1666777@gmail.com

Jiayu Shang  
Dept. of Electrical Engineering  
City University of Hong Kong  
Kowloon, Hong Kong SAR, China  
jyshang2-c@my.cityu.edu.hk

Yanni Sun  
Dept. of Electrical Engineering  
City University of Hong Kong  
Kowloon, Hong Kong SAR, China  
yannisun@cityu.edu.hk

September 7, 2022

ABSTRACT

Bacteriophages, which are viruses infecting bacteria, are the most ubiquitous and diverse entities in the biosphere. There is accumulating evidence revealing their important roles in shaping the structure of various microbiomes. Thanks to (viral) metagenomic sequencing, a large number of new bacteriophages have been discovered. However, lacking a standard and automatic virus classification pipeline, the taxonomic characterization of new viruses seriously lag behind the sequencing efforts. The high diversity, huge abundance, and limited number of characterized phages pose great challenges for taxonomic classification. Recently, several viral taxonomic classification methods have been proposed to tackle these challenges. Therefore, a comprehensive comparison and benchmarking are needed to establish for the state-of-the-art computational taxonomic classification of bacteriophages. In this work, we summarized the challenges and progress of phage taxonomic classification. Then we rigorously tested six recently published tools that demonstrated good performance in their publications. We demonstrated their utilities and tested them on multiple datasets, including the RefSeq dataset, short contigs, simulated metagenomic dataset, mock metagenomic datasets, and the latest genomes. This study provides a comprehensive review of phage taxonomic classification in different scenarios and practical guidance for choosing appropriate taxonomic classification pipelines.  
Contact: yannisun@cityu.edu.hk
1 Introduction

Bacteriophages (aka phages) are viruses that infect bacteria [1]. Phages are the most abundant biological entities on Earth. It is estimated that there are more than $10^{31}$ bacteriophages on the planet, outnumbering every other organism on Earth combined [2,3]. In most microbial communities, phages play a crucial role by shaping and maintaining microbial ecology [4,5], facilitating co-evolutionary relationships [6,7,8], and promoting microbial evolution through horizontal gene transfer [9,10].

Phages are diverse in size, morphology, and genomic organization [11,12]. They have a variety of structural morphologies, among which tailed double-stranded DNA (dsDNA) phages [13,14] are the most abundant. Besides dsDNA phages, there are also phages with single-stranded DNA (ssDNA) [15], single-stranded RNA (ssRNA) [16] or double-stranded RNA (dsRNA) [17]. Phages also have a wide range of genome sizes. The shortest phage genome is from Leuconostoc phage L5, with only 2,435 bp. Recently, an increasing number of megaphages (>200kb) have been sequenced, demonstrating unique genomic features [18]. Because of the high diversity of genomes, phages infecting different hosts typically have a low similarity. However, phages that infect the same host may also have considerable differences in their genomes [19,20].

Phages can live in different microbial ecosystems along with the colonization of their bacterial host. The first viral metagenome of uncultured marine viral communities was published in 2002 [21]. It is now demonstrated that phages can be found in a wide variety of environments, including aquatic ecosystems [22,23], human gut [24,25], and soil [26,11]. Phages can shape the composition and function of underlying ecosystems through two different lifestyles: temperate and virulent. Temperate phages will integrate their genomes into bacterial chromosomes and replicate with their host. They will maintain this state, which is also called prophages, until being induced by the environment’s condition, such as appropriate temperature and pH value. Then, temperate phages will enter the lytic cycle to kill the host [27,28]. In contrast, virulent phages do not integrate their genomes into the hosts. They stay in the lytic cycle and kill the hosts after replicating themselves [29].

The unique properties and life styles make phages key players in multiple applications. For example, phage therapy is a promising strategy for treating bacterial infections, particularly those with antibiotic-resistant bacteria. It has been found that intravenous phage preparations could treat Staphylococcus aureus that induced pneumonia in mice [30,31]. In addition, phages can be used to treat gastrointestinal infections. It has been demonstrated that phages are effective in reducing intestinal pathogens and have less impact on the composition of the intestinal microbiota compared to antibiotics [32,33,34,35]. Moreover, phages are important in food safety. The use of specific phage treatments in the food industry can prevent product spoilage and limit the spread of bacteria, providing a safe environment for animal and plant food production [36,37,38,39].

However, despite the abundance and importance of phages in various ecosystems, the understanding of phages is still very limited. According to the database supported by the National Center for Biotechnology Information (NCBI), the number of identified phages changed from 5,860 in 2015 to 14,819 in 2022 in the RefSeq database, which is tripled in size. Besides the reference genomes, there are roughly 10,004,207 assembled phages in the Genbank database in 2022, an almost tenfold increase compared to 2015 (1,893,134). However, the characterization of phages cannot keep pace with the fast increase of the sequencing data.

Assigning phages into different taxonomic groups is a fundamental step following phage discovery. The official taxonomy was established by the International Committee on Taxonomy of Viruses (ICTV) [40], which organizes viruses in several taxonomic levels, including order, family, subfamily, and genus. Within the ICTV, the Bacterial and Archaeal Viruses Subcommittee (BAVS) is responsible for the phages’ taxa. BAVS classifies phages based on a variety of phage properties, including the molecular composition of the genome (ss/ds, DNA, or RNA), the morphology, the structure of the capsid, and the host range [41]. Recently, with the increasing availability of viral genomes, using genomes for taxonomic classification has become more widely accepted [42]. Due to the extensive sequencing efforts for virus discovery, ICTV cannot catch up with the sheer number of newly identified phages, and thus many viruses are still not classified. One challenge behind this delay is the lack of standard, accurate, and comprehensive taxonomic classification tools for phages. Indeed, phage classification is not a trivial problem. High diversity, abundance, and limited known phages can pose significant challenges for taxonomic analysis. In addition, many newly discovered phages lack homologous genes to reference genomes, which can handicap alignment-based tools. This issue becomes more serious when only contigs, rather than complete or near complete genomes, can be assembled from metagenomic data because contigs may only contain partial ORF. Therefore, achieving accurate and comprehensive phage taxonomic classification remains a challenge.

To overcome these difficulties, several attempts have been made for automatic phage classification. These tools often have different designs and were tested on different datasets by their authors. Without a comprehensive comparison on the same set of data, it is difficult for users to choose the most appropriate solution for their needs. In this paper, we
present a comprehensive benchmark of the main players in phage taxonomic classification. The remaining of this review is organized as follows. First, we will describe the main methods/models for existing phage taxonomic classification approaches. Then, we rigorously evaluate six most widely used or newest approaches in different usage scenarios. In particular, we tested these tools on complete virus genomes, short contigs, simulated metagenomic dataset, and mock metagenomic datasets. In addition, we tested the ability of these tools in classifying new viruses, which were sequenced after the tools’ publication time. By comparing their performance and analyzing the underlying reasons, we draw conclusions and provide guidance for users about choosing the most appropriate tools for different scenarios.

2 Approaches for phage taxonomic classification

Most phage taxonomic classification approaches can output three ranks: order, family, and genus. In this review, we focus on comparing different tools’ performance at family level because of the following reasons. First, the taxonomy by ICTV is under constant changes, which affects genus more than family or order. For example, there are 735 genera in the ICTV database released in 2016. However, the number of genera increased to 2,224 in 2020. In contrast, the increase of the family number is from 122 in 2016 to 189 in 2020 [43]. The overhaul of the genus-level taxonomy can make the definition of “ground truth” ambiguous. Second, because classification at higher taxonomic ranks is usually easier than lower ranks due to the smaller inter-class similarities, evaluation at the order level is not challenging enough to compare different tools. In addition, Caudovirales, an order of phages known as the tailed phages, contains the majority of the total phage sequences (about 96%) [44] and can be classified by all of the tools mentioned above, we thus focus on the classification of the families belonging to Caudovirales in this work.

The phage taxonomic classification methods are summarized in Table 1, which includes a brief description, publication year, and the required input data type of each tool. A majority of these tools conduct phage taxonomic classification based on sequence comparison, utilizing nucleotide-level or protein-level similarity between a query virus and reference database. The comparison-based methods differ in their constructed reference database, the alignment method, and how they utilize these alignments. Both pairwise sequence alignment and hidden Markov model (HMM)-based profile alignments are commonly used. Multiple tools construct virus protein families and use them as marker genes. Using markers usually incurs less memory usage than using all phage genomes. But newly sequenced phages with novel genes may not be aligned to any marker gene families and thus cannot be assigned to a known class. Learning-based models have also been applied to phage classification. Learning models can automatically infer the sequence patterns in phage genomes of different families and use the learned features for automatic classification. Learning-based models have intrinsic advantages in classifying new or highly diverged phage sequences, which often lack detectable alignments against reference database. A more detailed description of these tools is provided below, following the chronological order in Table 1.

Phage Proteomic Tree [45, 46] is a relatively early program providing phage genome classification down to the family level. It extracts protein sequences from virus genomes and clusters these sequences using BLASTP [58]. Then the clusters in Phage Proteomic Tree are refined and scored. Finally, the alignment scores are converted to distances, which were used to generate the final tree using the neighbor-joining algorithm.

Taxon-specific signature genes can be identified in most virus taxa. POGs (Phage Orthologous Groups) [47] is a collection of clusters of orthologous genes from phages, presented as profiles (multiple sequence alignment). The viral families of POGs are filtered as ‘Viruses[Organism] NOT cellular organisms [ORGN] NOT srdb_refseq[PROP] AND vhost bacterial[filter] AND “complete genome”[All Fields]’ in NCBI. Signatures are extracted for each taxon, and we can use BLASTP to search for matches among the viral protein sequences. POGs are designed to be well suited for defining taxon-specific signature genes, and the profiles built from POGs are more sensitive and specific to search for signature genes in a given dataset.

GRAViTy [48, 49] also extract protein sequences from virus genomes and cluster these sequences using BLASTP [58]. GRAViTy generates protein profile hidden Markov models (PPHMMs) and genomic organization models (GOMs) based on the sequences from BLASTP-based clustering. Then it computes Composite Generalized Jaccard (CGJ) similarity scores (a geometric mean of the two generalized Jaccard scores computed for a pair of PPHMM signatures and a pair of GOM signatures) between each sequence pair to construct the heat map and dendrogram and estimate sequences relatedness.

CCP77 [50] applies a concatenated protein phylogeny for classification of tailed dsDNA viruses belonging to the specific order Caudovirales. Classiphage [51, 52] uses phage-specific Hidden Markov Models (HMMs) [59] profiles generated from clusters of related proteins for classification. The HMM profiles are built using the produced multi-sequence alignment files by the “hmmbuild” command. Classiphage 2.0 additionally trains an Artificial Neutral Network (ANN) using phage family-proteome to phage-derived HMMs scoring matrix, which can classify more phage families and include more features than its previous version. vConTACT [53, 54] is a high-throughput network-based approach...
Table 1: Overview of bioinformatic approaches used for phage taxonomic classification. We only focus on ranks above species.

| Name                | Year | Description                                                                 | Input data                  | Lowest level |
|---------------------|------|-----------------------------------------------------------------------------|-----------------------------|--------------|
| Phage Proteomic Tree [45][46] | 2002 | It uses the BLASTP distance and protein distance scores (similarity between two proteins) to generate phage proteomic trees, which can describe the relationships between different phages and can serve as a genome-based classification system for phages. | protein sequences           | Family       |
| POGs [47]           | 2013 | It provides a collection of orthologous genes clusters from phages, represented as profiles. It extracts virus-specific genes, and then classifies phages by aligning query sequences against the marker genes utilizing BLAST. | genome sequences            | Genus        |
| GraViTy [48][49]    | 2018 | It conducts taxonomic classification by computing sequence relatedness between viruses using Composite Generalized Jaccard (CGJ) distances that integrate homology detection outputs and shared genomic features. | genome sequences            | Genus        |
| CCP77 [50]          | 2019 | A Phylogeny-based taxonomic classification for Caudovirales, inferring a concatenated Caudovirales protein (CCP77) tree based on the concatenation of protein markers using a maximum-likelihood method. | genome sequences            | Genus        |
| ClassiPhage [51][52] | 2019 | It uses a set of phage-specific Hidden Markov Models (HMMs) generated from clusters of related proteins for phage taxonomic classification. Classiphage 2.0 adds an Artificial Neural Network (ANN) in the models. | protein sequences           | Family       |
| vConTACT [53][54]   | 2019 | A network-based application utilizing whole genome gene-sharing profiles, which integrates distance-based hierarchical clustering and confidence scores for virus classification. | protein sequences           | Genus        |
| CAT [55]            | 2019 | It can provide taxonomic classification for contigs or contig bins utilizing homology search for open reading frames (ORFs). | genome sequences            | Genus        |
| VPF-Class [56]      | 2021 | It automates the classification by assigning the proteins to a set of Viral Protein Families (VPFs), which are then used to estimate the similarity between query genomes with classified genomes. | genome sequences            | Genus        |
| PhaGCN [57]         | 2021 | A semi-supervised learning model. It formulates the taxonomic classification problem as a node classification problem in a knowledge network, which is constructed by combining the DNA sequence features and protein sequence similarity. | genome sequences            | Family       |
REVIEW

utilizing whole-genome gene-sharing profiles. It clusters the input viral genomes together with characterized genomes. The genomes in the same cluster indicate same family or genus, and the predicted family can be inferred if there are characterized genomes in the same cluster.

CAT [55] provides taxonomic classification using homology searches. It uses DIAMOND BLASTP to identify homologous sequences and then assigns query sequences into taxa with a voting approach. The authors of CAT show that using the best hit strategy can lead to low specificity and thus design a more robust strategy based on multiple hits. Users can select the reference database and tune the setting, which is more flexible than some other tools. Moreover, it has a very low memory usage.

VPF-Class [56] provides both taxonomic classification and host prediction for input viral genomes. It compares predicted proteins against the set of constructed Viral Protein Families (VPFs) (from the IMG/VR system). Then it derives taxonomic classifications and confidence scores from the list of VPFs detected on each query genome. VPF-Class does not require users to download and select the reference datasets, which simplifies the application.

PhaGCN [57] is a semi-supervised learning model for phage taxonomic classification. This model constructs a knowledge graph by combining the DNA sequence features learned by CNN and protein sequence similarity gained from the gene-sharing network. The learning model can incorporate the automatically learned features for each contig. However, unlike sequence comparison-based approaches, PhaGCN only accepts phage-like sequences as input. Thus, a pre-processing step is needed for detecting those contigs from metagenomic data. A number of tools such as VirFinder [60], Seeker [61] and PhaMer [62] can be applied in the pre-processing step.

3 Experiments and Results

Out of the tools included in Table 1, we focus on evaluating six of them: vConTACT 2.0, POGs, ClassiPhage, PhaGCN, CAT, and VPF-Class. These tools are recently published and demonstrated good performance in their own or others’ tests. In addition, the corresponding codes and tools are still under maintenance. None of them requires an internet connection or a web server. To mimic the scenario of applying these tools to datasets without known taxonomic composition, we apply all these tools with their default parameters, which are optimized by the authors. The commands for running all these tools are available in the Supplementary File. All the tools were run on Intel® Xeon® Gold 6258 R CPU with 8 cores.

3.1 Dataset

We rigorously evaluated these phages taxonomic classification tools on multiple datasets with increasing complexity. The detailed information is listed below.

The RefSeq dataset  This dataset contains all Caudovirales phage genomes released before March 2022 in RefSeq, which is a widely used benchmark dataset in the phage classification task. Totally, 4,270 phage genomes were downloaded. Table 2 shows the number of genomes within the 14 families under Caudovirales. More than half of them are from the Siphoviridae family. For the tools that need protein sequences as input, we used Prodigal [63] to predict and translate the genes into the proteins.

Short contigs dataset This dataset contains segments with different lengths, including 500 bp, 1,000 bp, 3,000 bp, 5,000 bp, 10,000 bp, and 15,000 bp. We randomly generated the segments from the reference genomes. For each length, we cut ten segments from each phage genome by selecting a random start position. Finally, we had 42,700 phage contigs for each length and 256,200 for all different lengths. Then, we used these segments to evaluate the performance of the six tools on short contigs.

Simulated metagenomic dataset We used a simulated metagenomic dataset generated by six common bacteria living in the human gut [62]. We utilized metaSPAdes [64] to assemble the reads into contigs. Then PhaMer [62] was applied to identify bacteriophages from metagenomic data, and the labels of the contigs were determined using BLAST [65]. Eventually, 76 contigs were used in the experiments. More details about this dataset will be provided in the later sections.

Mock metagenomic dataset Two mock community metagenomes were downloaded from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) with accession numbers SRR10417995 and DRR205589. Similar to the simulated metagenomic data, the reads were assembled into contigs, then phages-like contigs were identified and labeled using metagenomic assembly and virus detection programs. Finally, we had 6 and 8 phage contigs with known taxa labels from SRR10417995 and DRR205589, respectively.
### Table 2: The latest 14 families under Caudovirales from the RefSeq database. Number: the number of complete genomes in each family. ‘✓’: the family labels that can be returned by a tool.

| Family Name         | Number | POGs | CAT | ClassiPhage | vConTACT 2.0 | VPF-Class | PhaGCN |
|---------------------|--------|------|-----|-------------|--------------|-----------|--------|
| Siphoviridae        | 2217   | ✓    | ✓   | ✓           | ✓            | ✓         | ✓      |
| Myoviridae          | 864    | ✓    | ✓   | ✓           | ✓            | ✓         | ✓      |
| Autographiviridae   | 371    | ✓    |     | ✓           | ✓            | ✓         | ✓      |
| Podoviridae         | 233    | ✓    | ✓   | ✓           | ✓            | ✓         | ✓      |
| Herelleviridae      | 135    | ✓    |     | ✓           | ✓            | ✓         | ✓      |
| Drexlerviridae      | 116    | ✓    |     |             | ✓            | ✓         | ✓      |
| Demerecviridae      | 92     | ✓    |     |             | ✓            | ✓         |        |
| Schitoviridae       | 75     | ✓    |     |             | ✓            | ✓         |        |
| Ackermannviridae    | 63     | ✓    |     |             | ✓            | ✓         |        |
| Rountreevirida      | 35     | ✓    |     |             | ✓            | ✓         |        |
| Salasmavirida       | 34     |      |     |             | ✓            | ✓         |        |
| Chasevirida         | 14     | ✓    |     |             | ✓            |           |        |
| Zobellvirida        | 13     |      |     |             | ✓            |           |        |
| Guelinvirida        | 8      |      |     |             | ✓            |           |        |

### 3.2 Evaluating criteria for different tools

#### 3.2.1 The number of families

Although Caudovirales contains 14 families, not every tool can classify phages in all of them. In particular, ClassiPhage, POGs, and VPF-Class can only distinguish phages in three families: Siphoviridae, Myoviridae, and Podoviridae. PhaGCN can identify phages in eight families: Siphoviridae, Myoviridae, Drexlerviridae, Podoviridae, Demerecviridae, Autographiviridae, Ackermannviridae, and Herelleviridae. vConTACT 2.0 does not indicate the number of families that can be classified. According to the latest version tool we downloaded, the families of the classified viral genomes in vConTACT 2.0 are Siphoviridae, Myoviridae, Podoviridae, Ackermannviridae, and Herelleviridae. The number of families classified by CAT depends on the database files supplied by the user. We used the most recent version database files (20210107 version) provided by CAT, which can classify phages into nine families: Siphoviridae, Drexlerviridae, Podoviridae, Myoviridae, Demerecviridae, Autographiviridae, Ackermannviridae, Chaseviridae, and Herelleviridae. These methods can only predict limited phage families because the reference or the training datasets have limited labels. And usually, they did not provide an easy-to-use module to update the database. The families that are included by different tools are shown in Table 2. Only 3 families can be identified by all tools: Siphoviridae, Myoviridae, and Podoviridae. They constituted the initial classification scheme of tailed phages, and for a long time in the past, the vast majority of tailed phages were classified into these three families [66]. Therefore, in the first experiment on complete phage genomes, we not only show the tools’ performance on all Caudovirales phages but also present their performance on these three common families.

#### 3.2.2 Metrics

An ideal phage classification tool should assign correct labels for as many inputs as possible. But there is usually a tradeoff between the percentage of prediction and the accuracy of the prediction. Some tools may sacrifice the percentage of prediction in order to achieve high specificity and accuracy while others may predict more with lower accuracy. Thus the first metric is prediction rate, which is the ratio of outputs with assigned labels ($N_{\text{pred}}$ in Equation 1) to the total input ($N_{\text{all}}$ in Equation 1). Because some tools only provide a family name as output, commonly used metrics such as AUROC cannot be computed. In this work, we calculated accuracy, recall, precision, and F1-score for each tool (Equations 2-5).

- **TP** is the number of samples with correct predictions.
- **TN** is the number of samples with correct predictions.
- **FN** is the number of samples with incorrect predictions.
- **FP** is the number of samples with incorrect predictions.

$N_{\text{correct}}$ is the number of samples with correct predictions. $N_{\text{total}}$ is the total number of samples used to evaluate, which can be $N_{\text{pred}}$ or $N_{\text{all}}$ when we report accuracy for all samples ($N_{\text{all}}$) or for samples with predictions ($N_{\text{pred}}$), respectively. Providing accuracy for all samples has the advantage of using the same denominator (i.e. $N_{\text{all}}$) for all tools. But it penalizes the tools of low prediction rate twice. On the other hand, reporting accuracy for only samples with predictions removes the impact of prediction rate but may favor tools with low prediction rate (i.e. small $N_{\text{pred}}$). Thus, reporting both can provide a more comprehensive evaluation for users. $N_{\text{class}}$ is the total number of classes (i.e. $N_{\text{class}} = 14$). $w_i$ is the percentage of class $i$ in all samples ($N_{\text{all}}$), and all tools use the same $w_i$ list (e.g. $w_{\text{Siphoviridae}} = 2217/4270$). $TP_i$, $TN_i$, and $FN_i$ are the true positive, true negative, and false negative for...
class \(i\). Because the class that cannot be identified has a precision value of 0 (the TP value is 0), this class will not be counted in the final result.

\[
\text{Prediction rate} = \frac{N_{\text{pred}}}{N_{\text{all}}} \tag{1}
\]

\[
\text{Accuracy} = \frac{N_{\text{correct}}}{N_{\text{total}}} \tag{2}
\]

\[
\text{Precision} = \sum_{i=0}^{N_{\text{class}}} \text{Precision}_i w_i = \sum_{i=0}^{N_{\text{class}}} \frac{TP_i}{TP_i + FP_i} w_i \tag{3}
\]

\[
\text{Recall} = \sum_{i=0}^{N_{\text{class}}} \text{Recall}_i w_i = \sum_{i=0}^{N_{\text{class}}} \frac{TP_i}{TP_i + FN_i} w_i \tag{4}
\]

\[
\text{F1-score} = \sum_{i=0}^{N_{\text{class}}} \frac{2 \times \text{Precision}_i \times \text{Recall}_i}{\text{Precision}_i + \text{Recall}_i} w_i \tag{5}
\]

### 3.3 Classification performance on the RefSeq database

First, we compared the results of six tools on 4,270 complete phage genomes from RefSeq. The prediction rate of the tools on phage genomes is listed in Table 3, which reveals that almost all pipelines can assign family labels for more than 90% of phage genomes except CAT (62.2%). VPF-Class has the highest prediction rate on this dataset.

**Table 3: The prediction rate on complete genomes.**

| Tools          | prediction rate |
|----------------|-----------------|
| PhaGCN         | 0.955           |
| ClassiPhage    | 0.970           |
| VPF-Class      | 0.998           |
| vConTACT 2.0   | 0.974           |
| POGs           | 0.903           |
| CAT            | 0.622           |

A good taxonomic classification tool should have both a high prediction rate and high accuracy. Fig. 4(A) shows that the classification performance of all benchmarked tools on the RefSeq dataset (with respect to predicted samples) varies widely. The ranges of accuracy, precision, recall, and F1-score are 0.42-0.95, 0.46-0.92, 0.42-0.95, and 0.40-0.93, respectively. CAT achieves the highest accuracy (95.2%), and PhaGCN is the second best (92.3%). Fig. 4(B) shows that the performance of each tool on all samples is worse than that on predicted samples, which is expected because none of the tools achieves 100% prediction rate. For example, the prediction rate of CAT is lower than all the other tools, leading to its worse performance on all samples. PhaGCN can predict more phages (95.5%) at the expense of a slight decrease in precision (4.2%). Therefore, for users who are looking for high precision, CAT is recommended, and if they want to predict more phages with a better tradeoff between recall and precision, PhaGCN is a better choice.

Fig. 5 shows the recall/precision on predicted samples for three families (\textit{Siphoviridae}, \textit{Myoviridae}, and \textit{Podoviridae}), which all the tools can distinguish. The results reveal that PhaGCN, CAT, VPF-Class, and vConTACT 2.0 achieve a high recall on all three labels. What’s more, all approaches have high precision on \textit{Siphoviridae}, meaning they can better classify the phages in this family. However, the precision of these tools on \textit{Podoviridae} is lower than the other two families, suggesting that they tend to assign the genomes not belonging to \textit{Podoviridae} to this family. A closer look of those genomes reveals that they are mostly from families that cannot be classified by these tools, such as \textit{Schitoviridae}, \textit{Rountreeviridae}, \textit{Salasmaviridae}. Therefore, the lower precision on \textit{Podoviridae} is related to the large number of aforementioned families which can not be classified. The more families a tool can identify, the higher precision it can achieve. For example, the tools that can classify \textit{Autographiviridae} (CAT, PhaGCN) achieve higher precision on \textit{Podoviridae} than other tools. Overall, PhaGCN, CAT, VPF-Class, and vConTACT 2.0 have consistent recalls in classifying the genomes of these three families, and CAT performs the best.
Figure 1: The performance of each tool on 4,270 complete genomes from RefSeq. (A): the performance on predicted samples (the genomes that can be assigned taxa). (B): the performance on all samples (4,270 genomes). X-axis: the metrics. Y-axis: the values.

Figure 2: The classification recall and precision of three families: Siphoviridae, Myoviridae, and Podoviridae.

3.4 Classification performance on short contigs

We run all methods on the short contig dataset, and the performance comparison of all the tested tools is shown in Fig. 3. First, we recorded the prediction rate of each tool. As shown in Fig. 3(A), POGs and PhaGCN maintain a high prediction rate (>90%) on short sequences. Similar to 3.3, CAT has low prediction rates (around 60%) among different lengths. The prediction rates of VPF-Class and vConTACT 2.0 drop sharply when the length of the contigs is less than 3000 bp. vConTACT 2.0 cannot handle very short contigs because short contigs tend to contain few genes and thus cannot be connected with characterized genomes based on gene sharing significance. VPF-Class and ClassiPhage also have difficulties in classifying short contigs because of their dependence on hmmscan/hmmssearch \[67\], which may incur fewer or short/fragmented alignments for short inputs. Because PhaGCN only accepts contigs longer than 2000 bp, we do not show its results on 500 bp and 1000 bp in Fig. 3. VPF-Class has the highest prediction rate if the inputs are longer than 10kbp.

Fig. 3 (B) and (C) show the performance of the six tools on short fragments derived from RefSeq. The results on predicted samples shown in (B) reveal that the accuracy of all methods becomes better with the increase in sequence length. This is expected because longer sequences usually provide more information for classification. Similar to the results in 3.3, PhaGCN keeps excellent accuracy in both conditions. vConTACT 2.0, POGs, and VPF-Class have similar performances on predicted samples. CAT remains the best on predicted samples but performs worse on all samples because of its lower prediction rate. In addition, the performance of ClassiPhage, vConTACT 2.0, and VPF-Class drops significantly compared to other tools in (C), which is also due to the change in their prediction rates. The overall observation is that the classification ability of POGs, PhaGCN, and CAT is not significantly affected by the length of the contigs. Due to the length limitation of PhaGCN, it is not suitable for classifying contigs shorter than 2000 bp.
classifying contigs longer than 5000 bp, vConTACT 2.0, PhaGCN, and VPF-Class can be good choices. Similar to the results in 3.3, CAT is recommended if precision is the main consideration.

Figure 3: (A) The prediction rate of six tools on short fragments with different lengths. (B) The accuracy of six tools on the short fragments with predicted family labels. (C) The accuracy of six tools for all short fragments. X-axis: the length of contigs.

Table 4: Family composition of the simulated metagenomic dataset and two mock metagenomic datasets.

| Simulated metagenomic dataset          | Phage Family | Number | Phage Family | Number |
|----------------------------------------|--------------|--------|--------------|--------|
| Myoviridae                             |              | 55     | Siphoviridae | 12     |
| Ackermannviridae                       |              | 6      | Podoviridae  | 1      |
| Demerecviridae                         |              | 1      | Drexlervirida| 1      |
| SRR10417995                            | Phage Family | Number | Phage Family | Number |
| Podoviridae                            |              | 1      | Podoviridae  | 7      |
| Siphoviridae                           |              | 5      | Siphoviridae | 1      |
| total                                  |              | 6      | total        | 8      |

3.5 Classification performance on simulated metagenomic dataset

In this experiment, we used the simulated metagenomic dataset provided in PhaMer [62]. The dataset is a small-scale metagenomic dataset simulated by CAMISIM [58] using the commonly seen bacteria living in the human gut and the phages that infect these bacteria. The reads were assembled into contigs using metaSPAdes [64]. As shown by Fig. 3, the performance of most tools drops significantly for contigs with length below 3000 bp. Thus, the contigs shorter than 3000 bp were removed. To assign labels to the contigs, we used BLAST [65] to map contigs to reference genomes and calculated the coverage. Only the contigs with at least 90% of the sequence aligning to a reference genome were kept. Others are likely chimeric contigs due to assembly errors and thus are not used for testing. Finally, the number of contigs we can use in the experiment is 76. The name of the families and the number of genomes within each family are listed in Table 4. Compared to Table 2, this test set contains a different abundance distribution for the component families, which can thus change the observed performance of these tools.

Fig. 4 shows the performance of the six pipelines on the simulated metagenomic dataset. The comparison reveals that POGs and PhaGCN can classify almost all contigs, and the prediction rates of the other three approaches are around 90% except for CAT (21%). The performance of PhaGCN, vConTACT 2.0, POGs, and VPF-Class do not differ significantly from their performance on the RefSeq dataset. The good performance is expected because according to 3.4, these three tools can achieve high accuracy on the contigs longer than 3000 bp. CAT still has a low prediction rate but high specificity, which is consistent with the previous observations.
3.6 Classification performance on mock metagenomic datasets

After testing the approaches on the RefSeq database and the simulated datasets, we compared all tools on mock metagenomic datasets. Two mock metagenomic datasets, SRR10417995 and DRR205589, were downloaded from NCBI SRA. First, we used the FASTQC \[69\] to control the quality of the data and removed bad reads (low quality, too short, etc.) with FASTP \[70\]. Similar to \[3.5\], the cleaned reads of these two datasets were fed into metaSPAdes separately and were assembled into contigs. Because the real sequencing data might contain data not belonging to phages, we ran PhaMER to screen the phage-like contigs and removed the contigs with length <3,000 bp. Then, the output contigs were labeled by BLAST. The contigs with coverage and identity higher than 90% were used for comparison. Finally, the family name and the number of contigs used in the experiment are listed in Table 4.

We compared the phage labels assigned by PhaGCN, vConTACT 2.0, POGs, VPF-Class, and ClassiPhage on the assembled contigs. The prediction results of all methods are shown in Fig. 5 and 6, which reveal that the performance of all tools on the DRR205589 dataset is better than that on SRR10417995. On the one hand, it may be caused by some “erroneous” contigs assembled in the DRR205589 dataset. On the other hand, most contigs in SRR10417995 belong to Podoviridae and the majority of contigs in DRR205589 are Siphoviridae. It was demonstrated in \[3.3\] that these tools perform worse on Podoviridae than Siphoviridae, which could lead to the worse performance in SRR10417995. VPF-Class can classify all contigs correctly in both datasets and performs best among these tools. One possible reason is that the Viral Protein Families (VPFs) used in VPF-Class were generated from manually curated metagenomic viral
contigs. Moreover, all the approaches can classify most of the contigs on SRR10417905. This is expected because according to the results in 3.4, all tools can have a better performance on the contigs longer than 3,000 bp.

3.7 Classification performance on “new” genomes

Considering that the most important utility of the phage classification tool is to predict labels for new sequences, we evaluate this utility in this section. We first tested these tools on the genomes from RefSeq released before and after their publication years (e.g. POGs: before and after 2013, PhaGCN: before and after 2021). The results are shown in Fig. 7(A), which reveals that most of the tools perform better on pre-publication data except CAT and VPF-Class. However, a confounding factor here is that the number of post-publication samples is different for different tools. To mitigate this problem, we further show the performance of these tools on newly sequenced genomes in 2020 and 2021.

Specifically, we tested each tool on the latest genomes from the RefSeq database released in 2020 (1173 sequences) and 2021 (691 sequences), respectively. Fig. 7(B) and (C) show the accuracy on genomes released in different years compared with the accuracy on all samples ($N_{all}$ in Equation 1). The results reveal that the performance on the dataset from 2021 is better than that on all samples. Conversely, the accuracy on genomes released in 2020 is lower. PhaGCN and CAT do not have obvious differences between these two datasets, showing that they are robust in classifying genomes from different years. In contrast, the performance of the other four tools has a large fluctuation on the two datasets, suggesting that their performance depends on the test data. Therefore, this experiment shows that PhaGCN and CAT are robust for classifying new sequences. Using publishing/uploading date to separate training and test sequences is a commonly used strategy in machine learning for sequence analysis. The above experimental results also show that this method may suffer from some limitations. Newly sequenced genomes in different years can be biased depending on the sequencing projects. Thus, using only date-based test set may not be sufficient to evaluate the performance of different tools.

3.8 Comparison of Running Time

Running time is also an important factor to consider for practical usage. Table 5 shows the running time of the tools on the complete genome dataset containing 4,270 sequences. It reveals that most of them took hours to complete their run. It is important to note that most of them demand a lot of time on sequence alignment, including the learning-based tools. VPF-Class took the longest time to process the data, and ClassiPhage is the fastest tool. Currently, these tools’ classification performance don’t synchronize with their running time, demonstrating room for improvement.

Table 5: The total running time of tools for classifying 4,270 genomes. All the tools are run on Intel® Xeon® Gold 6258 R CPU with 8 cores.

| Tool            | Time (min) |
|-----------------|------------|
| PhaGCN          | 1538       |
| ClassiPhage     | 106        |
| CAT             | 340        |
| vConTACT 2.0    | 771        |
| POGs            | 1237       |
| VPF-Class       | 6410       |
4 Conclusion

In this work, we present a comprehensive benchmarking on phage taxonomic classification. We designed experiments to evaluate the performance of six state-of-the-art approaches among multiple datasets, including RefSeq, short contigs, simulated metagenomic dataset, and mock metagenomic datasets. We also evaluate their performance on the latest genomes. The experimental results show that PhaGCN has the best performance and stability for phage taxonomic classification. Therefore, we can infer that the approach utilizing machine learning models can gain a better classification result than the tools only relying on alignment. Because of the relatively low number of tools compared here, it may not mean that this is a generalizable observation. VPF-Class, vConTACT 2.0, CAT, and POGs have approximately equal classification results on the long sequences (>5000 bp). However, POGs and CAT perform considerably better on classifying short contigs (<3000 bp) than vConTACT 2.0 and VPF-Class. Combined with the results of the experiments in our work, CAT and POGs are more recommended to classify short contigs. PhaGCN, vConTACT 2.0, and VPF-Class are suitable to be used in long contigs classification (>5000 bp). Users can also make a choice according to their requirements (high prediction rate or high precision). VPF-Class has an excellent performance on metagenomic contigs. What’s more, PhaGCN has stable good performance in most conditions. If there is a need to classify genomes in multiple datasets, PhaGCN is the best choice.

We also recorded the running time of each tool. Combining the results of our experiments, there are still several limitations among current approaches, which can be roughly claimed into two points: first, the time and accuracy of these approaches can not go hand in hand. Almost all of the tools spend hours processing the RefSeq dataset. Therefore, it will be much more time-consuming to process large samples in the future, which is a challenge that needs to be overcome. Second, the current methods are limited in the number of different families they can predict, and most of them can only predict the largest number or most frequently used families. Therefore, phage taxonomic classification tools also need to update the predicted labels in time to cope with the possible emergence of more new families in the future. It seems that the challenges of phage taxonomic classification remain numerous. There is currently a lack of tools that can both classify phages taxonomy efficiently and closely follow the number of new phages taxa. The computational family-level taxonomic classification methods have been enormously valuable for advancing the understanding of phage diversity. We express our extreme gratitude to those that developed them. In the future, we hope that more and more effective approaches will be proposed to address the need for the continued refinement of phage taxonomic classification tools.

Data Availability

The detailed information of the code and datasets is provided in the supplementary file.

Funding

City University of Hong Kong (Project 9678241 and 7005453) and the Hong Kong Innovation and Technology Commission (InnoHK Project CIMDA).

References

[1] Stephen McGrath, D van Sinderen, et al. Bacteriophage: genetics and molecular biology. Caister Academic Press, 2007.
[2] Curtis A Suttle. Viruses in the sea. Nature, 437(7057):356–361, 2005.
[3] Scott LaFee and Heather Buschman. Novel phage therapy saves patient with multidrug-resistant bacterial infection. UC San Diego News Center, University of California, USA, 2017.
[4] Britt Koskella and Sean Meaden. Understanding bacteriophage specificity in natural microbial communities. Viruses, 5(3):806–823, 2013.
[5] T Frede Thingstad. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnology and Oceanography, 45(6):1320–1328, 2000.
[6] Ana Georgina Cobián Güemes, Merry Youle, Vito Adrian Cantú, Ben Felts, James Nulton, and Forest Rohwer. Viruses as winners in the game of life. Annual review of virology, 3:197–214, 2016.
[7] Lesley Hoyles, Anne L McCartney, Horst Neve, Glenn R Gibson, Jeremy D Sanderson, Knut J Heller, and Douwe van Sinderen. Characterization of virus-like particles associated with the human faecal and caecal microbiota. Research in microbiology, 165(10):803–812, 2014.
REVIEW

[8] Cynthia B Silveira and Forest L Rohwer. Piggyback-the-winner in host-associated microbial communities. npj Biofilms and Microbiomes, 2(1):1–5, 2016.

[9] Maryury Brown-Jaque, William Calero-Cáceres, and Maite Muniesa. Transfer of antibiotic-resistance genes via phage-related mobile elements. Plasmid, 79:1–7, 2015.

[10] Yin Ning Chiang, José R Penadés, and John Chen. Genetic transduction by phages and chromosomal islands: The new and noncanonical. PLoS pathogens, 15(8):e1007878, 2019.

[11] Cheryl-Emiliane T Chow and Curtis A Suttle. Biogeography of viruses in the sea. Annu Rev Virol, 2(1):41–66, 2015.

[12] Hans-W Ackermann. Classification of bacteriophages. The bacteriophages, 2:8–16, 2006.

[13] Kathryn M Kauffman, Fatima A Hussain, Joy Yang, Philip Arevalo, Julia M Brown, William K Chang, David VanNesbergh, Joseph Elsherbini, Radhey S Sharma, Michael B Cutler, et al. A major lineage of non-tailed dsdna viruses as unrecognized killers of marine bacteria. Nature, 554(7690):118–122, 2018.

[14] Jennifer R Brum, Ryan O Schenck, and Matthew B Sullivan. Global morphological analysis of marine viruses shows minimal regional variation and dominance of non-tailed viruses. The ISME journal, 7(9):1738–1751, 2013.

[15] Efrem S Lim, Yanjiao Zhou, Guoyan Zhao, Irma K Bauer, Lindsay Droit, I Malick Ndao, Barbara B Warner, Philip I Tarr, David Wang, and Lori R Holtz. Early life dynamics of the human gut virome and bacterial microbiome in infants. Nature medicine, 21(10):1228–1234, 2015.

[16] Tim Loeb and Norton D Zinder. A bacteriophage containing rna. Proceedings of the National Academy of Sciences, 47(3):282–289, 1961.

[17] Peter Mertens. The dsrna viruses. Virus research, 101(1):3–13, 2004.

[18] Yihui Yuan and Meiying Gao. Jumbo bacteriophages: an overview. Frontiers in microbiology, 8:403, 2017.

[19] Graham F Hatfull. Bacteriophage genomics. Current opinion in microbiology, 11(5):447–453, 2008.

[20] Mart Krupovic, David Prangishvili, Roger W Hendrix, and Dennis H Bamford. Genomics of bacterial and archaeal viruses: dynamics within the prokaryotic virome. Microbiology and molecular biology reviews, 75(4):610–635, 2011.

[21] Mya Breitbart, Peter Salamon, Bjarne Andresen, Joseph M Mahaffy, Anca M Segall, David Mead, Farooq Azam, and Forest Rohwer. Genomic analysis of uncultured marine viral communities. Proceedings of the National Academy of Sciences, 99(22):14250–14255, 2002.

[22] John H Paul, Matthew B Sullivan, Anca M Segall, and Forest Rohwer. Marine phage genomics. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 133(4):463–476, 2002.

[23] Burton Guttmann, Raul Raya, and Elizabeth Kutter. Basic phage biology. Bacteriophages: Biology and applications, 4:30–63, 2005.

[24] Thomas DS Sutton and Colin Hill. Gut bacteriophage: current understanding and challenges. Frontiers in Endocrinology, 10:784, 2019.

[25] Pilar Manrique, Michael Dills, and Mark J Young. The human gut phage community and its implications for health and disease. Viruses, 9(6):141, 2017.

[26] Kurt E Williamson, Jeffry J Fuhrmann, K Eric Wommack, Mark Radosevich, et al. Viruses in soil ecosystems: an unknown quantity within an unexplored territory. Annu Rev Virol, 4(1):201–219, 2017.

[27] Allan Campbell. The future of bacteriophage biology. Nature Reviews Genetics, 4(6):471–477, 2003.

[28] Cristina Howard-Varona, Katherine R Hargreaves, Stephen T Abedon, and Matthew B Sullivan. Lysogeny in nature: mechanisms, impact and ecology of temperate phages. The ISME journal, 11(7):1511–1520, 2017.

[29] Zack Hobbs and Stephen T Abedon. Diversity of phage infection types and associated terminology: the problem with ‘lytic or lysogenic’. FEMS microbiology letters, 363(7), 2016.

[30] Emilie Saussereau and Laurent Debarbieux. Bacteriophages in the experimental treatment of pseudomonas aeruginosa infections in mice. Advances in virus research, 83:123–141, 2012.

[31] Joseph M Ochieng’ Oduor, Nyamongo Onkoba, Fredrick Maloba, and Atunga Nyachieo. Experimental phage therapy against haematogenous multi-drug resistant staphylococcus aureus pneumonia in mice. African Journal of Laboratory Medicine, 5(1):1–7, 2016.

[32] Matthieu Gaillart, Luisa De Sordi, Damien Maura, Harindra Arachchi, Stevenn Volant, Marie-Agnès Dillies, and Laurent Debarbieux. Bacteriophages to reduce gut carriage of antibiotic resistant uropathogens with low impact on microbiota composition. Environmental microbiology, 18(7):2237–2243, 2016.
[33] Janet Y Nale, Janice Spencer, Katherine R Hargreaves, Anthony M Buckley, Przemysław Trzepiński, Gillian R Douce, and Martha RJ Clokie. Bacteriophage combinations significantly reduce clostridium difficile growth in vitro and proliferation in vivo. Antimicrobial agents and chemotherapy, 60(2):968–981, 2016.

[34] Abhishek Jaiswal, Hemanta Koley, Amit Ghosh, Anup Palit, and Banwarilal Sarkar. Efficacy of cocktail phage therapy in treating vibrio cholerae infection in rabbit model. Microbes and Infection, 15(2):152–156, 2013.

[35] Beatriz Gutiérrez and Pilar Domingo-Calap. Phage therapy in gastrointestinal diseases. Microorganisms, 8(9):1420, 2020.

[36] Sanna M Sillankorva, Hugo Oliveira, and Joana Azeredo. Bacteriophages and their role in food safety. International journal of microbiology, 2012, 2012.

[37] P Garcia, B Martinez, JM Obeso, and A Rodriguez. Bacteriophages and their application in food safety. Letters in applied microbiology, 47(6):479–485, 2008.

[38] Brid Coffey, Susan Mills, Aidan Coffey, Olivia McAuliffe, R Paul Ross, et al. Phage and their lysins as biocontrol agents for food safety applications. Annu. Rev. Food Sci. Technol., 1:449–468, 2010.

[39] Diana Gutiérrez, Lorena Rodríguez-Rubio, Lucía Fernández, Beatriz Martínez, Ana Rodríguez, and Pilar García. Applicability of commercial phage-based products against listeria monocytogenes for improvement of food safety in spanish dry-cured ham and food contact surfaces. Food Control, 73:1474–1482, 2017.

[40] Michael J Adams, Elliot J Lefkowitz, Andrew MQ King, Balázs Harrach, Robert L Harrison, Nick J Knowles, Andrew M Kropinski, Mart Krupovic, Jens H Kuhn, Arcady R Mushegian, et al. 50 years of the international committee on taxonomy of viruses: progress and prospects. Archives of virology, 162(5):1441–1446, 2017.

[41] Moira B Dion, Frank Oechslin, and Sylvain Moineau. Phage diversity, genomics and phylogeny. Nature Reviews Microbiology, 18(3):125–138, 2020.

[42] Elliot J Lefkowitz, Donald M Dempsey, Robert Curtis Hendrickson, Richard J Orton, Stuart G Siddell, and Donald B Smith. Virus taxonomy: the database of the international committee on taxonomy of viruses (ictv). Nucleic acids research, 46(D1):D708–D717, 2018.

[43] Grete Francesca Privitera, Salvatore Alaimo, Alfredo Ferro, and Alfredo Pulvirenti. Virus finding tools: current solutions and limitations. Briefings in Bioinformatics, 23(4):bbac235, 2022.

[44] H-W Ackermann. 5500 phages examined in the electron microscope. Archives of virology, 152(2):227–243, 2007.

[45] Forest Rohwer and Rob Edwards. The phage proteomic tree: a genome-based taxonomy for phage. Journal of bacteriology, 184(16):4529–4535, 2002.

[46] Yosuke Nishimura, Takashi Yoshida, Megumi Kuronishi, Hideya Uehara, Hiroyuki Ogata, and Susumu Goto. Viptree: the viral proteomic tree server. Bioinformatics, 33(15):2379–2380, 2017.

[47] Pakorn Aiewsakun and Peter Simmonds. The genomic underpinnings of eukaryotic virus taxonomy: creating a sequence-based framework for family-level virus classification. Microbiome, 6(1):1–24, 2018.

[48] Pakorn Aiewsakun, Evelien M Adriaenssens, Rob Lavigne, Andrew M Kropinski, and Peter Simmonds. Evaluation of the genomic diversity of viruses infecting bacteria, archaea and eukaryotes using a common bioinformatic platform: steps towards a unified taxonomy. The Journal of general virology, 99(9):1331, 2018.

[49] Soo Jen Low, Mária Džunková, Pierre-Alain Chaumeil, Donovan H Parks, and Philip Hugenholtz. Evaluation of a concatenated protein phylogeny for classification of tailed double-stranded dna viruses belonging to the order caulovirales. Nature microbiology, 4(8):1306–1315, 2019.

[50] Cynthia Maria Chibani, Anton Farr, Sandra Klama, Sascha Dietrich, and Heiko Liesegang. Classifying the unclassified: a phage classification method. Viruses, 11(2):195, 2019.

[51] Cynthia Maria Chibani, Florentin Meinecke, Anton Farr, Sascha Dietrich, and Heiko Liesegang. Classiphages 2.0: Sequence-based classification of phages using artificial neural networks. BioRxiv, page 558171, 2019.

[52] Benjamin Bolduc, Ho Bin Jang, Guilhem Doulcier, Zhi-Qiang You, Simon Roux, and Matthew B Sullivan. vcontact: an ivirus tool to classify double-stranded dna viruses that infect archaea and bacteria. PeerJ, 5:e3243, 2017.

[53] Ho Bin Jang, Benjamin Bolduc, Olivier Zablocki, Jens H Kuhn, Simon Roux, Evelien M Adriaenssens, J Rodney Brister, Andrew M Kropinski, Mart Krupovic, Rob Lavigne, et al. Taxonomic assignment of uncultivated prokaryotic virus genomes is enabled by gene-sharing networks. Nature biotechnology, 37(6):632–639, 2019.
[55] FA von Meijenfeldt, Ksenia Arkhipova, Diego D Cambuy, Felipe H Coutinho, and Bas E Dutilh. Robust taxonomic classification of uncharted microbial sequences and bins with cat and bat. *Genome biology*, 20(1):1–14, 2019.

[56] Joan Carles Pons, David Paez-Espino, Gabriel Riera, Natalia Ivanova, Nikos C Kyprides, and Mercè Llabrés. VPF-Class: taxonomic assignment and host prediction of uncultivated viruses based on viral protein families. *Bioinformatics*, 2021.

[57] Jiayu Shang, Jingzhe Jiang, and Yanni Sun. Bacteriophage classification for assembled contigs using graph convolutional network. *Bioinformatics*, 37(Supplement_1):i25–i33, 2021.

[58] Stephen F Altschul, Thomas L Madden, Alejandro A Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J Lipman. Gapped blast and psi-blast: a new generation of protein database search programs. *Nucleic acids research*, 25(17):3389–3402, 1997.

[59] Sean R Eddy. Accelerated profile hmm searches. *PLoS computational biology*, 7(10):e1002195, 2011.

[60] Jie Ren, Kai Song, Chao Deng, Nathan A Ahlgren, Jed A Fuhrman, Yi Li, Xiaohui Xie, Ryan Poplin, and Fengzhu Sun. Identifying viruses from metagenomic data using deep learning. *Quantitative Biology*, 8(1):64–77, 2020.

[61] Noam Auslander, Ayal B Gussow, Sean Benler, Yuri I Wolf, and Eugene V Koonin. Seeker: alignment-free identification of bacteriophage genomes by deep learning. *Nucleic acids research*, 48(21):e121–e121, 2020.

[62] Jiayu Shang, Xubo Tang, Ruocheng Guo, and Yanni Sun. Accurate identification of bacteriophages from metagenomic data using transformer. *arXiv preprint arXiv:2201.04778*, 2022.

[63] Doug Hyatt, Gwo-Liang Chen, Philip F LoCascio, Miriam L Land, Frank W Larimer, and Loren J Hauser. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC bioinformatics*, 11(1):1–11, 2010.

[64] Sergey Nurk, Dmitry Meleshko, Anton Korobeynikov, and Pavel A Pevzner. metaspades: a new versatile metagenomic assembler. *Genome research*, 27(5):824–834, 2017.

[65] Christian Camacho, George Coulouris, Vahram Avagyan, Ning Ma, Jason Papadopoulos, Kevin Bealer, and Thomas L Madden. Blast+: architecture and applications. *BMC bioinformatics*, 10(1):1–9, 2009.

[66] H-W Ackermann. Frequency of morphological phage descriptions in 1995. *Archives of virology*, 141(2):209–218, 1996.

[67] Simon C Potter, Aurélien Luciani, Sean R Eddy, Youngmi Park, Rodrigo Lopez, and Robert D Finn. Hmmer web server: 2018 update. *Nucleic acids research*, 46(W1):W200–W204, 2018.

[68] Adrian Fritz, Peter Hofmann, Stephan Majda, Eik Dahms, Johannes Dröge, Jessika Fiedler, Till R Lesker, Peter Belmann, Matthew Z DeMaere, Aaron E Darling, et al. Camisim: simulating metagenomes and microbial communities. *Microbiome*, 7(1):1–12, 2019.

[69] Simon Andrews et al. Fastqc: a quality control tool for high throughput sequence data. 2010.

[70] Shifu Chen, Yanqing Zhou, Yaru Chen, and Jia Gu. fastp: an ultra-fast all-in-one fastq preprocessor. *Bioinformatics*, 34(17):i884–i890, 2018.