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A novel phage from periodontal pockets associated with chronic periodontitis

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Abstract

Bacteriophages often constitute the majority of periodontal viral communities, but phages that infect oral bacteria remain uncharacterized. Here, we present the genetic analysis of the genome of a novel siphovirus, named Siphoviridae_29632, which was isolated from a patient with periodontitis using a viral metagenomics-based approach. Among 43 predicted open reading frames (ORFs) in the genome, the viral genes encoding structural proteins were distinct from the counterparts of other viruses, although a distant homology is shared among viral morphogenesis proteins. A total of 28 predicted coding sequences had significant homology to other known phage ORF sequences. In addition, the prevalence of Siphoviridae_29632 in a cohort of patients with chronic periodontitis was 41.67%, which was significantly higher than that in the healthy group (4.55%, $P < 0.001$), suggesting that this virus as well as its hosts may contribute to the ecological environment favored for chronic periodontitis.

Keywords Bacteriophage · Siphovirus · Periodontitis · Viral metagenomics · Case–control study

Introduction

The human oral cavity accommodates a large and diverse population of viruses that may be conditionally involved in oral diseases [1]. Periodontitis is highly prevalent among adults [2] and is characterized by inflammation around the teeth. Some investigators have suggested that a host immune response to specific pathogens may cause periodontitis [3, 4].

There is a large population of viruses in the oral cavity. However, given the higher abundance of bacterial cells than eukaryotic cells in the oral cavity, it is currently understood that the viruses residing in saliva and subgingival and supragingival biofilms mainly consist of bacteriophages, which are viruses that only infect bacteria [5–10]. Many phages can be classified into the order *Caudovirales*, including three families which are determined by their tail morphology. They are *Siphoviridae* (long and non-contractile, generally lysogenic with relatively narrow host ranges), *Myoviridae* (contractile, typically lytic with relatively broad host ranges), and *Podoviridae* (short and non-contractile,

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typically lytic with relatively narrow host ranges) [11, 12]. As phages can be found wherever their bacterial hosts are present [6, 13], their presence in the oral cavity is highly likely.

Phage-encoded proteins may increase virulence either directly through the effect of toxins and other virulence factors [14] or indirectly by facilitating bacterial fitness in the environment. Thus, screening of lysogenic bacteriophages is helpful in determining the virulence of many pathogens. In addition, the high host specificity of bacteriophages for certain strains of bacteria, particularly currently unculturable species, could probably help to reverse the course of bacterial infection by identifying phage-encoded proteins.

Bacteriophages may be involved in selecting the composition and gene transfer of bacterial communities to impact the microbial population [15]. In addition, interactions between bacteria and viruses may contribute to a broad diversity of bacteria in the local environment as suggested by various ecological models such as constant-diversity dynamics, periodic selection dynamics, and kill-the-winner dynamics [16–20]. However, our knowledge about the identities of viruses and bacteriophages in the oral cavity and their impact on the oral bacterial community remains insufficient [21–23], although several bacteriophages have been isolated from a number of oral bacteria [21, 23–27]. Though the oral bacterial community and its impact on human oral health have been analyzed by a few studies [6, 13, 28], the isolation of phages from the human mouth is rare [29].

In this study, a new bacteriophage, Siphoviridae_29632 (GenBank accession number: KY053532), was characterized from the oral cavity of a human periodontitis patient.

Methods

This study was reviewed and approved by the Ethics Committee of the Ninth People's Hospital School of Medicine (No. 201406). All investigations were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all of the recruited subjects.

Identification of a novel bacteriophage from human periodontal tissue

Samples were collected from 48 patients with severe periodontitis who visited the Ninth People's Hospital School of Medicine, Shanghai Jiao Tong University, China. Biopsy specimens were obtained from the periodontal pockets, gingival epithelium, and connective tissue facing the sulcus during periodontal surgery. Clinical data, including age, gender, bleeding on probing (BOP), pocket depth (PD), clinical attachment loss (CAL), plaque index (PLI), gingival index (GI), and alveolar bone resorption upon pantomography, were recorded for all participants. The inclusion and exclusion criteria used in this study are listed in Table 1.

Total nucleic acids were extracted from the biopsied tissues using the QIAamp viral RNA extraction kit (Qiagen). Viral particles in the collected samples were concentrated by filtration with the 0.45-μm filter and nuclease treatment with a mixture of DNases (Turbo DNase from Ambion, MA, USA, Baseline-ZERO from Epicentre, IL, USA, and benzonase from Novagen, Darmstadt, Germany) and RNase (Thermo Fisher Scientific, MA, USA) at 37 °C for 90 min before the nucleic acids were extracted [30–33]. Viral DNA and RNA libraries were constructed [30–32]. The libraries were then sequenced using the MiSeq platform. Paired-end reads of 250 bp were debarcoded using vendor software from Illumina. Clonal reads were removed and low sequencing quality tails were trimmed using Phred quality score ten as the threshold [33]. Trimmed sequences from each group were assembled into contigs by Sequencher software (version 5.4, Gene Codes Corporation, Ann Arbor, MI, USA), with a criterion of more than 95% identity over 35 bp to merge two fragments [34]. Singlet sequences and assembled contigs were then compared to the virus nucleotide database in NCBI virus reference proteome (ftp://www.ncbi.nih.gov/refseq/release/viral/) using the BLASTn and BLASTx methods with an E-value cutoff of $< 10^{-5}$, as described previously [31–34]. 1551 novel viral contigs were identified that resembled

Table 1 Criteria used to recruit patients for this study

Inclusion	Exclusion
i. Good oral health status	i. Alcoholism
ii. Teeth ≥ 20	ii. Major symptoms of malignancy
iii. No oral mucosal diseases	iii. Cardiovascular disease
iv. No removable dentures	iv. Chronic liver or kidney disorders
v. No orthodontic appliances	v. Diabetes
vi. Mean pocket depth ≥ 6 mm	vi. Human immunodeficiency virus infection
vii. Clinical attachment loss ≥ 3 mm	vii. Pregnancy
viii. Bleeding on probing	viii. Heavy cigarette smoking (> 15 cigarettes/day)
ix. Alveolar bone resorption	ix. Previous antibiotic intake (last 6 months)
	x. Previous periodontal treatment (last 6 months)

bacteriophage Siphovirus_contig89 (accession number: KF594194.1). The resultant contigs of the novel phage were assembled and mapped, and the sequencing data obtained with Geneious (version 8.0, New Zealand) using the low sensitivity and fastest parameter. The polymerase chain reaction (PCR) primers were then designed using the assembled sequence (primer sequences shown in supplementary Table S1), and the resultant PCR amplicons were sequenced by the Sanger method to confirm the newly identified phage genome. The final reaction volume was 50 µL, including 3 µL of extracted DNA, each primer at 10 pmol, and 25 µL of PrimerSTAR Max Premix (TaKaRa, Dalian, China). The PCR parameters were 3 min at 94 °C, followed by 30 s at 94 °C, 30 s at 55 °C, and 50 s at 72 °C for 35 cycles, with a final extension of 5 min at 72 °C. PCR amplicons were analyzed by electrophoresis in 1% agarose gels, then cloned, and sequenced.

Sequence analysis

BLASTn was first utilized to identify any known matches via gene homology in GenBank by comparing each nucleotide query sequence against the nucleotide sequence database. Next, the identity of the matched sequences was uncovered by TBLASTx. Candidate viral hits were compared to an in-house non-virus non-redundant protein database to remove false-positive viral hits using NCBI virus reference proteome (ftp://www.ncbi.nih.gov/refseq/release/viral/) [33]. Individual putative genes in the sequences were ascertained with the aid of open reading frame (ORF) Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/) [22] and Clusters of Orthologous Groups of proteins (http://www.ncbi.nlm.nih.gov/COG). Sequences with E values $< 10^{-5}$ were considered homologs. The putative functions of the translated proteins from the predicted ORFs were suggested through comparing the known protein sequences using the GenBank database and the BLASTp program. The GC content in the viral genome was calculated using the program Lasergene SeqMan, version 7.0 (DNAStar, USA). The E value of each read was scored and accordingly assigned to a single best-matching reference viral protein.

Phylogenetic analysis

The inferred protein sequences with described functions were selected for phylogenetic analysis, including the counterparts of other bacteriophages with ClustalX. Using the Molecular Evolutionary Genetics Analysis program (MEGA, version 4.0, USA), maximum likelihood trees generated with ORF amino acid p-distances and 1000 bootstrap replicates were plotted with the neighbor-joining method [35]. Bootstrap values were labeled at each branching point. Homology analysis was also conducted using the MEGA program, and it was expressed as a percentage.

Epidemiological investigation

To identify a potential link between the presence of the novel phage and chronic periodontitis, a case–control study was conducted at the Ninth People's Hospital, Shanghai, China. The study included 218 subjects, consisting of 108 subjects with chronic periodontitis and 110 subjects without periodontitis (mean age of the subjects: 40 ± 11.78 years old). Each participant was subjected to a comprehensive oral examination. The same selection criteria and sampling method were applied as described in the first part of the Methods section. This newly identified phage was detected by PCR assays with negative controls. The primer sequences were as follows: F, 5′-ATGAACCTCGGAGTC ATTACTCGC-3′; R, 5′-CGTAGACCTCGTTCAGCATCA CGT-3′. The cycling conditions were the same as described above. The presence frequency (%) of the new phage was calculated.

Statistical analysis was performed with SPSS software (version 20.0; IBM Corporation, Armonk, NY, USA). χ^2 tests were performed to examine differences of the two groups in gender distribution, BOP, and the prevalence of Siphoviridae_29632. Differences in age and periodontal indexes (PD, GI, PLI, and CAL) between groups were analyzed by the Student's t test.

Results

Genome characterization

The new phage falls into the *Siphoviridae* family of the order *Caudovirales*. The source patient from whom this novel bacteriophage was characterized was a 35-year-old male with alveolar bone resorption and bleeding on probing (sample number 42), and his PD, CAL, PLI, and GI values of sampling tooth were 11.4, 5.3, 2.7, and 1.8, respectively.

The newly identified phage genomic DNA is 29,632 bp long. Primers were designed to sequence the genome, and the results confirmed the phage genomic sequence. This new phage was designated as Siphoviridae_29632. The genomic sequence (GenBank accession number: KY053532) was analyzed and functionally annotated. It has a GC content of 59.64%. A total of 43 putative ORFs that aligned with

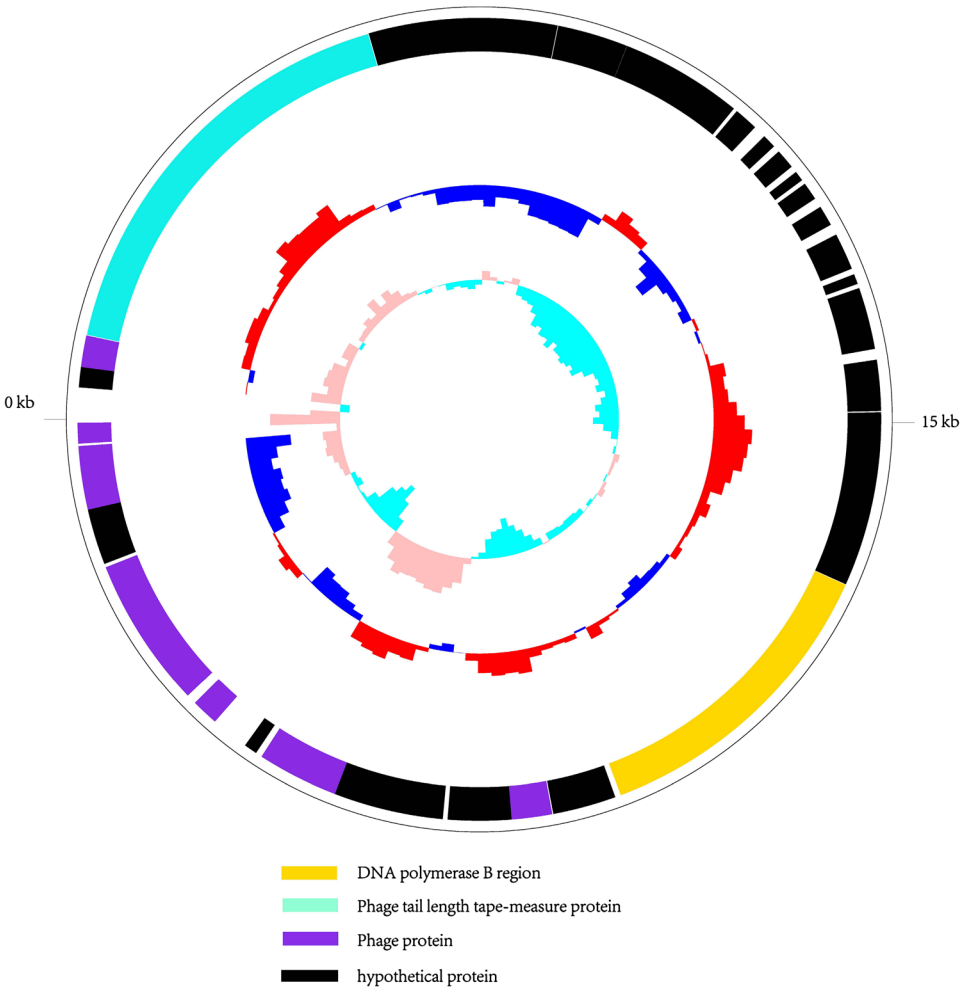

Fig. 1 The genomic organization of Siphoviridae_29632. Annotations and illustrations were made using glimmer 3.0 (code table = 11)

recognizable homologs in GenBank were identified in both strands of the Siphoviridae_29632 genome (Fig. 1). Of the 43 predicted ORFs, 28 of them exhibit significant homology to other known phage ORF sequences. 11 of the ORFs resembled Siphovirus_contig89. The identified sequences included those predicted to encode proteins with functions of phage holin, phage tail tape measure protein, DNA replication, and several glycoproteins (Table 2).

Phylogenetic analysis of Siphoviridae_29632

ORF3, ORF23, and ORF42 were predicted to encode phage tail length tape measure protein, DNA polymerase B, and glycoprotein gp106, respectively. The inferred amino acid sequences of these proteins were phylogenetically compared to those from other bacteriophages using the programs ClustalX and MegAlign.

In the phylogenetic tree with the tape measure protein sequences, Siphoviridae_29632 is grouped with the *Streptomyces* phage Rima (GenBank accession no. AOZ64977), the *Streptomyces* phage Olympic Helado (GenBank accession no. AOZ64888), and the *Arthrobacter* phage CapnMurica (GenBank accession no. ALY08626), all of which are members of the Siphoviridae family (Fig. 2). Amino acid sequence analysis revealed that tape measure protein shares 48.3% and 31.5% identity with the OKZ68507.1 and AOZ64888.1 strains, respectively (Table 3). The DNA polymerase gene in ORF23 matched Siphovirus_contig89 (Fig. 3) and shares 55.2% identity with gp072 protein (GenBank accession no. AIE38368.1) (Table 3). Gp106 in Siphoviridae_29632 shares the highest amino acid identity (62.2%) with the counterpart in Siphovirus_contig89 (GenBank accession no. AIE38387.1), and 46.3% identity with HTH DNA-binding domain protein

Table 2 Siphoviridae_29632 ORFs, gene products, and functional assignments

ORF	aa length	Matches	Accession number	*E* value	Identities (%)	Predicted function
1	79	Methyl-accepting chemotaxis protein [*Tepidibacter formicigenes*]	WP_072887681.1	2.9	40	Hypothetical protein
2	126	Uncharacterised protein [uncultured *Clostridium* sp.][a]	SCH18585.1	5.00E−36	47	Phage protein
3	1696	Phage tail tape measure protein [*Clostridiales bacterium* 41_21_two_genomes][a]	OKZ50087.1	2.00E−136	45	Phage tail length tape measure protein
4	306	MULTISPECIES: phage tail family protein [*Clostridiaceae*][a]	WP_006780986.1	1.00E−50	36	Hypothetical protein
5	442	Hypothetical protein [Siphovirus contig89][a]	AIE38348.1	7.00E−53	38	Hypothetical protein
6	279	Hypothetical protein [[*Clostridium*] leptum][a]	WP_003529585.1	2.00E−35	35	Hypothetical protein
7	278	Putative uncharacterized protein [*Eubacterium* sp. CAG:603][a]	CCZ04610.1	5.00E−20	26	Hypothetical protein
8	102	Phage holin [Siphovirus contig89][a]	AIE38350.1	8.00E−29	48	Hypothetical protein
9	102	Phage holin [Siphovirus contig89][a]	AIE38350.1	1.00E−29	66	Hypothetical protein
10	94	Hypothetical protein [Siphovirus contig89][a]	AIE38351.1	8.00E−28	58	Hypothetical protein
11	54	No match				Hypothetical protein
12	77	No match				Hypothetical protein
13	39	TIGR03985 family CRISPR-associated protein [*Hapalosiphon* sp. MRB220]	WP_053458350.1	0.7	39	Hypothetical protein
14	70	M23 family peptidase [*Idiomarina xiamenensis*]	WP_008487696.1	0.4	45	Hypothetical protein
15	80	PREDICTED: histone-lysine *N*-methyltransferase, H3 lysine-36 and H4 lysine-20 specific [Vicugna pacos]	XP_015090351.1	8.9	37	Hypothetical protein
16	146	Hypothetical protein PBI_GRAVY_53 [Gordonia phage Gravy][a]	AVO25293.1	2.00E−13	34	Hypothetical protein
17	39	No match				Hypothetical protein
18	245	Hypothetical protein [[*Clostridium*] scindens][a]	WP_024738798.1	6.00E−43	35	Protein of Unknown Function
19	201	gp067 [Siphovirus contig89][a]	AIE38360.1	2.00E−42	42	Hypothetical protein
20	310	gp069 [Siphovirus contig89][a]	AIE38363.1	7.00E−72	42	Protein of Unknown Function
21	208	Hypothetical protein C817_04328 [Dorea sp. 5-2]	EOS75805.1	3.6	38	Hypothetical protein
22	173	gp071 [*Rhodococcus* phage ReqiPoco6][a]	YP_009012652.1	8.00E−13	33	Hypothetical protein
23	1235	gp072 [Siphovirus contig89][a]	AIE38368.1	0	54	DNA polymerase B region
24	123	No match				Hypothetical protein
25	84	DNA-binding transcriptional regulator KdgR [Providencia rettgeri]	WP_042844205.1	3.9	41	Hypothetical protein
26	44	No match				Hypothetical protein
27	159	Hypothetical protein [*Subdoligranulum* sp. 4_3_54A2FAA][a]	WP_009325070.1	1.00E−29	40	Phage protein
28	175	Signal transduction histidine kinase [*Sinosporangium album*]	SDH02171.1	3	29	Hypothetical protein
29	77	DUF433 domain-containing protein [*Curtobacterium ammoniigenes*]	WP_083527665.1	1.4	31	Hypothetical protein
30	81	Transcriptional regulator [*Streptomyces viridochromogenes*]	WP_048587524.1	0.69	32	Hypothetical protein

Table 2 (continued)

ORF	aa length	Matches	Accession number	*E* value	Identities (%)	Predicted function
31	198	Hypothetical protein AUI47_08430 [*Acidobacteria* bacterium 13_1_40CM_2_68_5]	OLD63699.1	0.1	38	Hypothetical protein
32	42	No match				Hypothetical protein
33	121	Hypothetical protein [*Nocardia ignorata*][a]	WP_084476896.1	9.00E−07	32	Hypothetical protein
34	321	Hypothetical protein HMPREF0995_02220 [*Lachnospiraceae* bacterium 7_1_58FAA][a]	EHO33754.1	2.00E−92	47	Phage protein
35	51	Hypothetical protein [*Clostridium* sp. SS2/1][a]	WP_008390902.1	1.00E−11	63	Hypothetical protein
36	106	Hydrolase [*Streptomyces* phage Bing][a]	AVD99506.1	2.00E−38	63	Phage protein
37	126	gp105 [Siphovirus contig89][a]	AIE38386.1	2.00E−52	62	Phage protein
38	472	gp106 [Siphovirus contig89][a]	AIE38387.1	0	68	Phage protein
39	99	gp106 [Siphovirus contig89][a]	AIE38387.1	3.00E−24	76	Hypothetical protein
40	75	Phage tail protein [*Bacillus thuringiensis*][a]	PEY46257.1	1.00E−15	58	Protein of unknown function
41	69	Phage tail protein [*Bacillus thuringiensis*][a]	PEY46257.1	2.00E−15	80	Hypothetical protein
42	251	gp106 [Siphovirus contig89][a]	AIE38387.1	1.00E−97	63	Phage protein
43	81	Hypothetical protein CAPNMURICA_88 [Arthrobacter phage CaptnMurica][a]	ALY08688.1	1.00E−19	56	Phage protein

[a]Significant matches

from the *Arthrobacter* phage (GenBank accession no. YP_009300783.1) (Fig. 4). Collectively, these findings indicate that the novel phage is more closely related to the Siphoviridae family than to other families.

Investigation of the prevalence of Siphoviridae_29632

No difference in age or gender was noted between chronic periodontitis subjects and periodontally healthy subjects ($P = 0.124$ and 0.635, respectively). The mean pocket depth, clinical attachment loss, plaque index, and gingival index values of the two groups are shown in Table 4. PCR was used to detect the presence of the new phage. Forty-five (41.75%) of the 108 subjects in the periodontitis group and 5 (4.55%) of the 110 control subjects tested positive for Siphoviridae_29632 ($P < 0.001$) (Table 5). One gel image of PCR amplicons with the negative control is shown in supplementary Fig. S1.

Discussion

Viral metagenomics is a useful tool to characterize diverse viruses including phages [36]. Novel phages can be identified by comparing unknown sequences with known ones in a metagenomic database [37]. This identified phage, Siphoviridae_29632, was predicted as a new member of the *Siphoviridae* family, under the *Caudovirales* order. The new viral genomic DNA is 29,632 bp long, comparable to that of Siphovirus_contig89, which consists of 23,546 bp (accession number: KF594194.1).

All putative ORFs in the phage genomic DNA were analyzed using BLASTp analysis in the GenBank database. ORF3 encodes a tail length tape measure protein that is important in assembling and determining the length of the phage tail [38, 39]. In fact, sequences encoding other known phage structural proteins, including the capsid, tail tube, portal, and tail fiber, were not characterized in the Siphoviridae_29632 genome, which may be due to the lack of annotation data. Overall, Siphoviridae_29632 shares a high level of homology with other members of

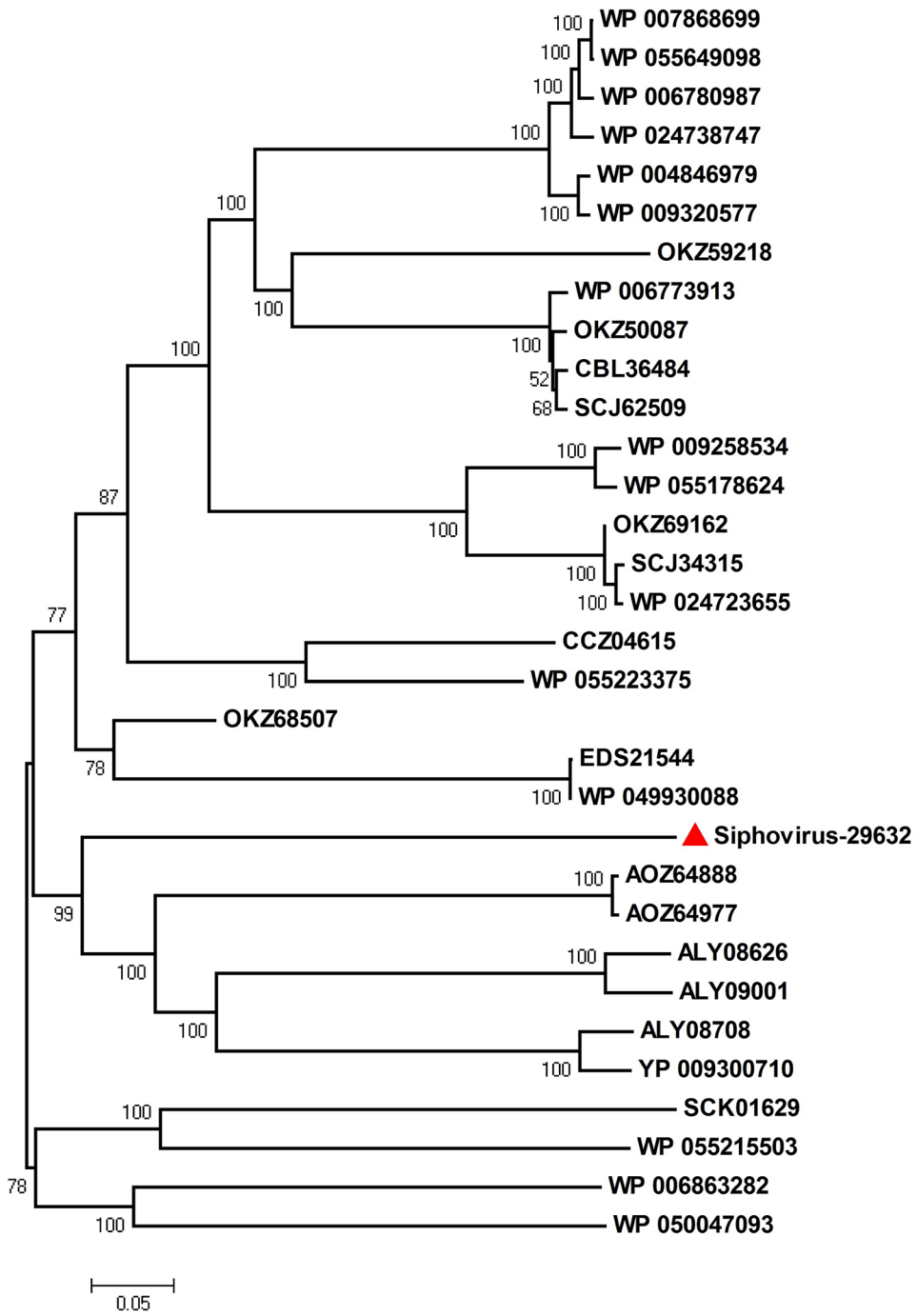

Fig. 2 Phylogenetic analysis of ORF3 amino acids. The tree was constructed using the neighbor-joining method with amino acid *p*-distances and 1000 bootstrap replicates in MEGA software, version 4.0 (USA). The solid triangle indicates the novel virus

the Siphoviridae family, including the *Streptomyces* phage Olympic Helado, the bacteriophage Siphovirus_contig89, and the *Arthrobacter* phage CapnMurica. However, phylogenetic analysis showed that Siphoviridae_29632 was evolutionarily distinct from other phage isolates, including Siphovirus_contig89.

A total of 28 of the 43 predicted ORFs contain sequences similar to the counterparts from other phages. Potential functions were only described for two of these recognizable proteins as the majority of them are functionally unknown [11]. In addition, interpretation of the data

Table 3 Sequence identity of Siphoviridae_29632 with other strains

	ORF3 referred sequence genbank accession no.	Siphovirus-29632, amino acids, %	ORF23 referred sequence genbank accession no.	Siphovirus-29632, amino acids, %	ORF42 Referred sequence genbank accession no.	Siphovirus-29632, amino acids, %
1	ALY08626.1	0.270	AIE38368.1	0.552	YP_009300783.1	0.463
2	ALY08708.1	0.313	AIE38454.1	0.551	YP_009017720.1	0.412
3	ALY09001.1	0.277	ALY08654.1	0.497	YP_009012687.1	0.408
4	AOZ64888.1	0.315	ALY08747.1	0.507	SFU33103.1	0.400
5	AOZ64977.1	0.315	ALY09029.1	0.496	SCJ63091.1	0.385
6	CCZ04615.1	0.295	CUP20432.1	0.543	SCJ35048.1	0.403
7	EDS21544.1	0.276	KKQ06471.1	0.550	OKZ69141.1	0.399
8	OKZ68507.1	0.483	OKZ50049.1	0.525	OKZ59144.1	0.415
9	OKZ69162.1	0.282	OKZ93630.1	0.525	EDS21562.1	0.341
10	SCJ34315.1	0.269	SCH17287.1	0.546	CUQ77095.1	0.336
11	SCJ62509.1	0.292	SCJ36459.1	0.539	CCY98945.1	0.328
12	WP_009258534.1	0.268	SCJ63720.1	0.526	AOZ64951.1	0.414
13	WP_009320577.1	0.291	WP_003500577.1	0.526	ALY09063.1	0.413
14	WP_049930088.1	0.276	YP_009012653.1	0.536	ALY08687.1	0.413
15	YP_009300710.1	0.289	YP_009017686.1	0.535	AIE38387.1	0.622

can be difficult because of the limited knowledge about the novel virus [29].

Periodontitis is a chronic oral disease that can lead to early tooth loss. The etiology for periodontal disease remains unclear, despite a high incidence. Infections with bacteria and/or viruses probably contribute to the pathogenesis of this disease. Recent studies have suggested their possible involvement with bacteriophages [13]. For instance, oral phages are frequently found in periodontally healthy subjects, and the presence of some lytic phages may be favored by periodontitis [5].

In this study, the prevalence of Siphoviridae_29632 infection was 41.67% (45/108) in the periodontitis group, which was significantly higher than that in the control group ($P < 0.001$) (Table 5), suggesting an association between periodontitis and this new phage. Phages in the oral cavity may act as a shaper of microbial community [23, 40], constituting the ecology of the human oral microbiome. Previous studies of oral bacteriophages have identified phages that parasitize oral pathogens such as *Aggregatibacter actinomycetemcomitans* [41, 42]. The presence of oral *A. actinomycetemcomitans* phages positively correlated with rapidly destructive periodontitis [43], suggesting that oral phages could be cytopathic. However, other studies have shown no links between phages and periodontal diseases [6, 44]. Therefore, the involvement of phages in oral diseases remains under debate. Additionally, some studies have suggested that the bacterial abundance does not always predict the relative amounts of their phages [5, 6]. An inverse relationship between the relative abundances of a putative host and the phage can be observed at most time points. Conversely, it has been shown that the amounts of phages for the genera *Streptococcus* and *Neisseria* positively reflect the abundances of their hosts [6]. Different abundance relationships between the host bacteria and their phages may depend upon the replication and release/lytic cycles of the phage.

Lytic oral phages and their cellular hosts can reach a dynamic equilibrium, and the phages are permanent members of the human oral microbiome [45]. Oral bacteriophages may evade the host's immune system and cause persistent infection through various mechanisms, which include but are not limited to encoding their own restriction/modification enzymes [46] and avoiding cognate sequences for cellular restriction/modification systems [46–48]. The high specificity of phages for certain bacterial strains can potentially be used to reverse the course of infection by targeting the host bacteria. For instance, phage M102 can be removed by selectively targeting mutant streptococci from dental plaques through disruption of the glucose side chain of the rhamnose glucose polysaccharide that is essential for phage M102 adsorption to *Streptococcus* mutants [49]. Some studies argue against treating mild or severe periodontal disease with antibiotics because these drugs can broadly affect

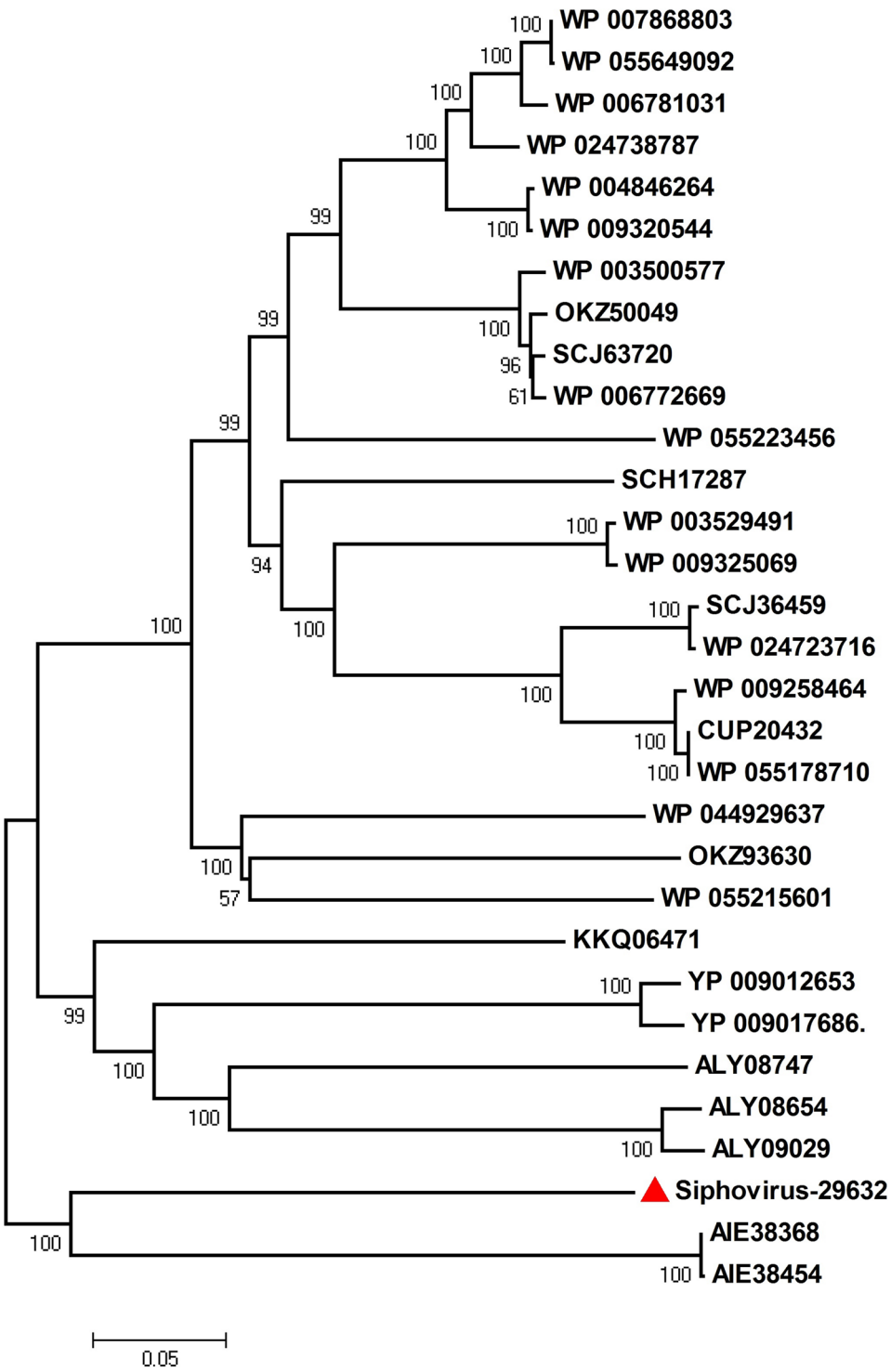

Fig. 3 Phylogenetic analysis of ORF23 amino acids. The tree was constructed using the neighbor-joining method with amino acid *p*-distances and 1000 bootstrap replicates in MEGA software, version 4.0 (USA). The solid triangle indicates the novel virus

an entire microbial community and trigger the selection of drug-resistant bacteria. Bacteriophages can be utilized to control pathogenic bacteria through lysis of the host cells during phage replication [50]. Renewed interest in phage therapy has translated basic studies of phages to experimental investigation of phage-based therapeutic applications [29, 51]. Phage-based treatment of periodontal disease has the potential to become a reality.

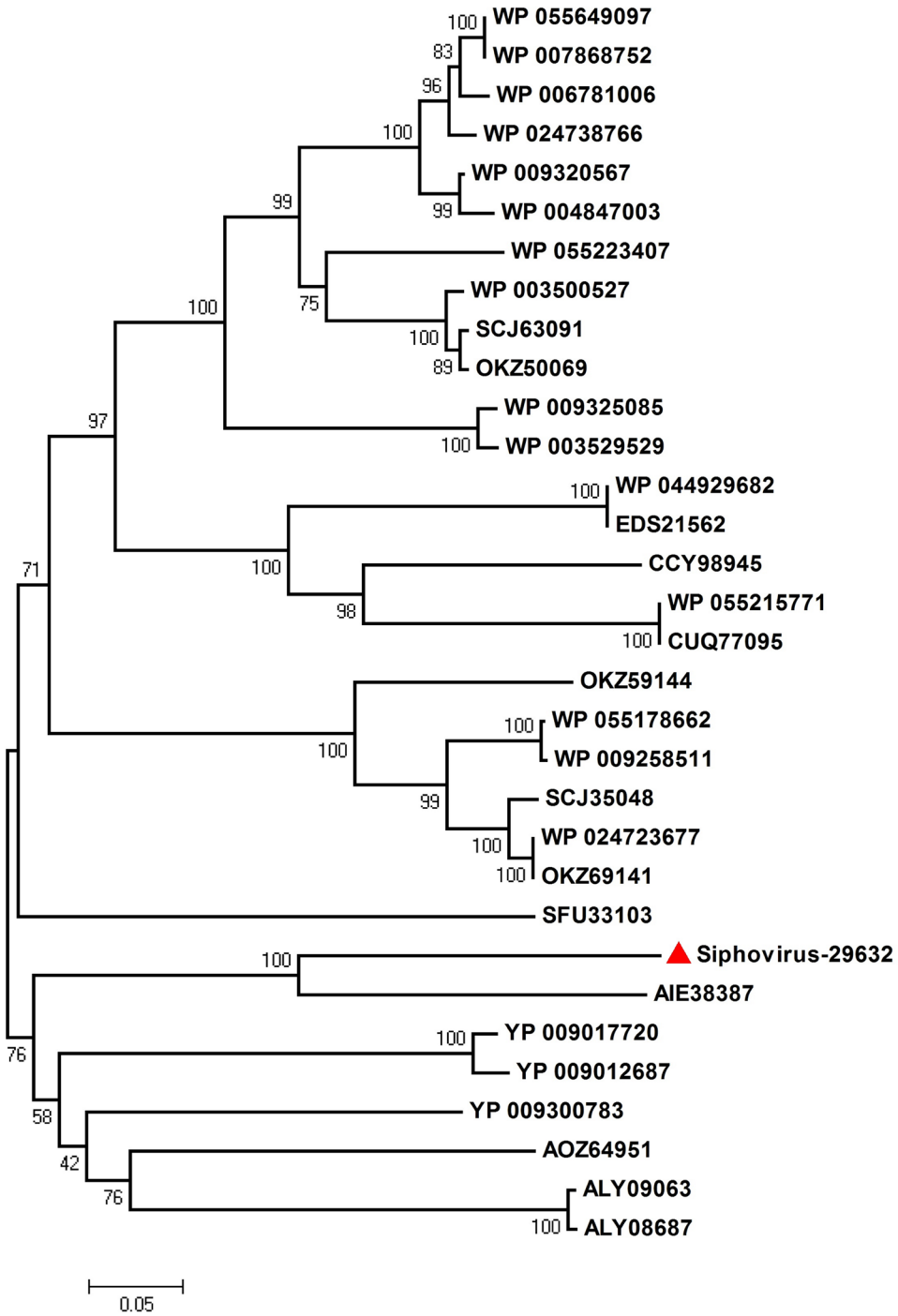

Fig. 4 Phylogenetic analysis of ORF42 amino acids. The tree was constructed using the neighbor-joining method with amino acid *p*-distances and 1000 bootstrap replicates in MEGA software, version 4.0 (USA). The solid triangle indicates the novel virus

Conclusions

In this study, we reported the genetic analysis and prevalence of a new phage in the periodontal environment, Siphoviridae_29632 virus. According to viral sequence analysis, this newly identified phage belongs to the Siphoviridae family. Siphoviridae_29632 presence was significantly higher in the chronic periodontitis group than in the healthy group. This finding may link this phage with chronic periodontitis. However, the full genome of this

Table 4 Comparison of lab findings between the periodontitis and control groups

Clinical parameter	Periodontitis (mean ± SD)	Control (mean ± SD)	P^a
Pocket depth	6.29 ± 3.41	1.80 ± 1.12	< 0.001
Clinical attachment loss	3.90 ± 1.56	0.12 ± 0.18	< 0.001
Gingival index	2.36 ± 0.55	0.17 ± 0.19	< 0.001
Plaque index	2.55 ± 0.46	0.33 ± 0.27	< 0.001

Obtained by the Student's *t* test (two groups)

SD standard deviation

[a]With the significance level set to $P < 0.05$

Table 5 Siphoviridae_29632 detection in the periodontitis and healthy groups

	Periodontitis group *N* (%)	Control group *N* (%)	Total	*P*
Siphoviridae_29632-positive	45 (41.67%)	5 (4.55%)	50 (22.94%)	
Siphoviridae_29632-negative	63 (58.33%)	105 (95.45%)	168 (77.06%)	< 0.001
Total	108 (100.00%)	110 (100.0%)	218 (100.0%)	

novel phage and its involvement in the periodontal environment remain to be determined.

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Author contributions XPF and TLS designed the study. XTD and ED completed the data analysis and statistics. YZ and FL completed the sample collection and the amplifications of the newly discovered full-length human anellovirus. YZ, TY, and XC completed the epidemiological investigation. All of the authors have read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethics Committee of the Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University approved this study (No. 201406).

Informed consent Informed consent was obtained from all individual participants included in the study.

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