Abstract: Escherichia phage N4 was isolated in 1966 in Italy and has remained a genomic orphan for a long time. It encodes an extremely large virion-associated RNA polymerase unique for bacterial viruses that became characteristic for this group. In recent years, due to new and relatively inexpensive sequencing techniques the number of publicly available phage genome sequences expanded rapidly. This revealed new members of the N4-like phage group, from 33 members in 2015 to 115 N4-like viruses in 2020. Using new technologies and methods for classification, the Bacterial and Archaeal Viruses Subcommittee of the International Committee on Taxonomy of Viruses (ICTV) has moved the classification and taxonomy of bacterial viruses from mere morphological approaches to genomic and proteomic methods. The analysis of 115 N4-like genomes resulted in a huge reassessment of this group and the proposal of a new family “Schitoviridae”, including eight subfamilies and numerous new genera.

Keywords: N4; Schitoviridae; virus taxonomy; ICTV; bacteriophages; bacterial viruses

1. Introduction

Escherichia phage N4 is a virulent phage that was originally isolated by Gian Carlo Schito from sewers in Genoa (Italy) in 1966 [1]. TEM analysis revealed a 70-nm-diameter capsid and a short tail, the characteristic features of a podovirus. Its genome consists of double-stranded DNA and has a length of 70,153 bp with about 400 bp direct repeats and short 3′-noncohesive extensions [2]. Analysis of the N4 genome and its replication revealed unique characteristics. Apart from a phenomenon called lysis-inhibition [3] that causes delayed lysis and a subsequently increased burst size upon infection, further analysis revealed a gene for a large virion-associated RNA polymerase (vRNAP) that became a characteristic for N4-like genomes and the use of in total three DNA-dependent RNA polymerases for transcription that were subject to different scientific questions and thus were intensively studied (Figure 1) [4]. The vRNAP is injected into the host cell along with DNA [5]. N4 genome is transcribed in three different temporal stages [6].
From a taxonomic perspective, phage N4 has a long history; from the first proposal in 1987 to establish a species Escherichia phage N4 [8] and its subsequent renaming to *Escherichia virus N4* in 2015, it has persisted as a genomic orphan. At the time of writing (Master Species List #35, ratified March 2020), its genus is now called *Enquatrovirus* and consists of only one species, representing four isolates.

Since 2015, following a comprehensive analysis of at that time 33 N4-like genomes [9], the number of publicly available N4-like phage genomes has nearly tripled [10]. The last report of the Bacterial and Archaeal Viruses Subcommittee [11] presented the new taxonomic classifications and reassessments that were achieved in 2018 and 2019 and listed a new order (*Tubulavirales*), ten new families, 22 new sub-families, 424 new genera and 964 new species, which still represent only a fraction of the genomes currently available. However, it has to be taken into account that ICTV does not classify viral strains or variants, i.e., those phage isolates with genomes that show ≥95% DNA sequence identity with an exemplar isolate of a species [12]. With regard to N4-like viruses, i.e., viruses encoding the vRNAP, only a rather small number of those have been officially classified by the ICTV so far. Currently, they are classified in 10 genera (*Baltimorevirus*, *Enquatrovirus*, *Gamaleyavirus*, *Ithacavirus*, *Johnsonvirus*, *Jwalphavirus*, *Litunavirus*, *Luzseptimavirus*, *Mukerjeevirus* and *Shizishanvirus*). This study provides further insight into the diversity and taxonomy of N4-like viruses using different approaches like genome-based phylogeny for deeper classification.

2. Results

2.1. Description of N4-Like Viruses

We downloaded 115 genomes from the NCBI databases (INSDC) [13,14] related to the N4-like group of viruses (Table 1). All N4-like phages and members of the proposed new family share the following characteristics:

- Podovirus morphology
- Genome size of 59–80 kb
- Linear genome with defined ends (terminal repeats expected)
- Presence of three RNA polymerase genes, including a large (~3500 aa) virion-associated RNA polymerase (vRNAP)
Table 1. List of N4-like genomes proposed to belong to the new family “Schitoviridae” available from INSDC databases.

| Phage                                      | Subfamily                 | Genus                      | Reference |
|--------------------------------------------|---------------------------|----------------------------|-----------|
| Achromobacter phage JWAlpha                | “Rothmandenesvirinae”     | Jwalphavirus               | [15]      |
| Achromobacter phage JWDelta               | “Rothmandenesvirinae”     | Jwalphavirus               | [15]      |
| Achromobacter phage phiAxp–3              | “Rothmandenesvirinae”     | “Dongdastvirus”            | [16]      |
| Achromobacter phage vB_AxyP_19–32_Axy04   | “Rothmandenesvirinae”     | “Pourcelvirus”             | [17]      |
| Achromobacter phage vB_AxyP_19–32_Axy10   | “Rothmandenesvirinae”     | “Pourcelvirus”             | [17]      |
| Achromobacter phage vB_AxyP_19–32_Axy11   | “Rothmandenesvirinae”     | “Pourcelvirus”             | [17]      |
| Achromobacter phage vB_AxyP_19–32_Axy12   | “Rothmandenesvirinae”     | “Dongdastvirus”            | [17]      |
| Achromobacter phage vB_AxyP_19–32_Axy13   | “Rothmandenesvirinae”     | “Inbricusvirus”            | [17]      |
| Achromobacter phage vB_AxyP_19–32_Axy22   | “Rothmandenesvirinae”     | “Pourcelvirus”             | [17]      |
| Achromobacter phage vB_AxyP_19–32_Axy24   | “Rothmandenesvirinae”     | “Dongdastvirus”            | [17]      |
| Acinetobacter phage Presley               | “Presleyvirus”            |                            | [18]      |
| Acinetobacter phage VB_ApiP_XC38          | “Dendorenvirus”           |                            | [20]      |
| Delftia phage RG–2014                     | “Rhodovirinae”            | Baltimorevirus             | [21]      |
| Dinoroseobacter phage DFL12phi1           | “Rhodovirinae”            | “Sanualagenivirus”          | [22]      |
| Dinoroseobacter phage DS–1410Ws–06        | “Rhodovirinae”            | Baltimorevirus             | [22]      |
| Enterobacter phage EcP1                   | “Enquatrovirinae”         | Enquatrovirus              | [3]       |
| Erwinia phage Ea9–2                      | “Erskinevirinae”          | Johnsonvirus               | [23]      |
| Erwinia phage phiEaP–8                    | “Erskinevirinae”          | Yonginvirus                | [24]      |
| Erwinia phage vB_EamP_Frozen              | “Erskinevirinae”          | Johnsonvirus               | [24]      |
| Erwinia phage vB_EamP_Gutmeister          | “Erskinevirinae”          | Johnsonvirus               | [24]      |
| Erwinia phage vB_EamP_Rexella             | “Erskinevirinae”          | Johnsonvirus               | [24]      |
| Erwinia phage vB_EamP-S6                  | “Enquatrovirinae”         | Gamaleyavirus              | [25]      |
| Escherichia phage Bp4                     | “Enquatrovirinae”         | Gamaleyavirus              | [26]      |
| Escherichia phage EC1–UPM                 | “Enquatrovirinae”         | Gamaleyavirus              | [27]      |
| Escherichia phage ECP1                    | “Enquatrovirinae”         | Gamaleyavirus              | [27]      |
| Escherichia phage IME11                   | “Enquatrovirinae”         | Gamaleyavirus              | [28]      |
| Escherichia phage OLB145                  | “Enquatrovirinae”         | Gamaleyavirus              | [28]      |
| Escherichia phage PD38                    | “Enquatrovirinae”         | Gamaleyavirus              | [29]      |
| Escherichia phage PGN829,1                | “Enquatrovirinae”         | Gamaleyavirus              | [30]      |
| Escherichia phage phi G17                 | “Enquatrovirinae”         | Gamaleyavirus              | [31]      |
| Escherichia phage PMBT57                  | “Enquatrovirinae”         | Gamaleyavirus              | [31]      |
| Escherichia phage Pollock                 | “Humphriesvirinae”        | “Pollockvirus”             | [32]      |
| Escherichia phage ST1IPh5                 | “Enquatrovirinae”         | Gamaleyavirus              | [32]      |
| Escherichia phage vB_EcoP_3HA13           | “Enquatrovirinae”         | Gamaleyavirus              | [32]      |
| Escherichia phage vB_EcoP_G7C             | “Enquatrovirinae”         | Gamaleyavirus              | [32]      |
| Escherichia phage vB_EcoP_PhaPEC5         | “Enquatrovirinae”         | Gamaleyavirus              | [33]      |
| Escherichia phage vB_EcoP_PhaPEC7         | “Enquatrovirinae”         | Gamaleyavirus              | [34]      |
| Klebsiella phage K8                       | “Enquatrovirinae”         | “Kaypocatirus”             | [34]      |
| Klebsiella phage KpCHEMY26                | “Humphriesvirinae”        | “Pylavivirus”              | [35]      |
| Klebsiella phage Pylas                    | “Humphriesvirinae”        | “Pylavivirus”              | [36]      |
| Pectobacterium phage Nepra                | “Humphriesvirinae”        | “Pylavivirus”              | [37]      |
| Pectobacterium phage phiA41               | “Humphriesvirinae”        | “Pylavivirus”              | [38]      |
| Pectobacterium phage vB_PaiP_CB1           | “Enstroivirus”            | “Chunavirus”               | [39]      |
| Pectobacterium phage vB_PaiP_CB3           | “Enstroivirus”            | “Chunavirus”               | [39]      |
| Pectobacterium phage vB_PaiP_CB4           | “Enstroivirus”            | “Chunavirus”               | [39]      |
| Pseudaloteromonas phage pYD6–A            | “Fuhrmanvirinae”          | “Mazavirius”               | [40]      |
| Pseudomonas phage 98FFluR60PP             | “Migularvirinae”          | “Littlefixvirus”           | [41]      |
| Pseudomonas phage DL64                    | “Migularvirinae”          | Lituavirus                  | [42]      |
| Pseudomonas phage inbricus                | “Rothmandenesvirinae”     | “Inbricusvirus”            | [43]      |
| Pseudomonas phage KPP21                   | “Migularvirinae”          | Lumixativirus               | [44]      |
| Pseudomonas phage LIT1                    | “Migularvirinae”          | Lituavirus                  | [45]      |
| Phage                                      | Subfamily          | Genus           | Reference |
|--------------------------------------------|--------------------|-----------------|-----------|
| Pseudomonas phage Littlefix                | "Migulavirinae"    | "Littlefixvirus"| [44]      |
| Pseudomonas phage LP14                     | "Migulavirinae"    | "Litunavirus"   |           |
| Pseudomonas phage LUX7                    | "Migulavirinae"    | "Luzseptimavirus"| [43]   |
| Pseudomonas phage LY218                   | "Migulavirinae"    | "Litunavirus"   |           |
| Pseudomonas phage Pa2                     | "Migulavirinae"    | "Litunavirus"   |           |
| Pseudomonas phage PA26                    | "Migulavirinae"    | "Litunavirus"   | [45]      |
| Pseudomonas phage PEV2                    | "Migulavirinae"    | "Litunavirus"   | [43]      |
| Pseudomonas phage phiCDa                  | "Migulavirinae"    | "Shizishanvirus"|           |
| Pseudomonas phage phi176                  | "Migulavirinae"    | "Litunavirus"   |           |
| Pseudomonas phage phiCDa                  | "Migulavirinae"    | "Litunavirus"   | [46]      |
| Pseudomonas phage vB_Pae1396P-5            | "Migulavirinae"    | "Litunavirus"   |           |
| Roseobacter phage RD–1410W1–01            | "Rhodovirinae"     | "Aouquinvirus"  | [21]      |
| Roseobacter phage RD–1410Ws–07             | "Rhodovirinae"     | "Sanyabayvirus" | [21]      |
| Roseovarius Plymouth podovirus 1          | "Rhodovirinae"     | "Plymouthvirus" | [53]      |
| Roseovarius sp. 217 phage 1               | "Rhodovirinae"     | "Plymouthvirus" | [53]      |
| Ruegeria phage vB_RpoP–V12                | "Rhodovirinae"     | "Aorunvirus"    |           |
| Ruegeria phage vB_RpoP–V13                | "Rhodovirinae"     | "Pomerovirus"   |           |
| Ruegeria phage vB_RpoP–V14                | "Rhodovirinae"     | "Aorunvirus"    |           |
| Ruegeria phage vB_RpoP–V17                | "Rhodovirinae"     | "Aorunvirus"    |           |
| Ruegeria phage vB_RpoP–V21                | "Rhodovirinae"     | "Aorunvirus"    |           |
| Salmonella phage FSL SP–058               | "Humphriesvirinae" | "Ithacavirus"   | [54]      |
| Salmonella phage FSL SP–076               | "Humphriesvirinae" | "Ithacavirus"   | [54]      |
| Shigella phage pSB–1                      | "Enquatrovirinae"  | "Gamalevirus"   | [55]      |
| Silicibacter phage DSS3phi2              | "Rhodovirinae"     | "Aorunvirus"    | [56]      |
| Sinorhizobium phage ort11                | "Huelavirinae"     | "Huclavirus"    | [57]      |
| Stenotrophomonas phage Pokken             | "Pulmoavirus"      | "Pokkenvirus"   | [58]      |
| Sulfotobacter phage EE36phi1              | "Rhodovirinae"     | "Aorunvirus"    | [56]      |
| Sulfitobacter phage phiCB2047-B           | "Rhodovirinae"     | "Rainefjordvirus"| [59]  |
| Vibrio phage 1.025.O._10N.222.46.B6        | "Pontosvirinae"    | "Nahantvirus"   | [60]      |
| Vibrio phage 1.026.O._10N.222.49.C7       | "Pontosvirinae"    | "Nahantvirus"   | [61]      |
| Vibrio phage 1.097.O._10N.286.49.B3       | "Pontosvirinae"    | "Dorisvirus"    | [62]      |
| Vibrio phage 1.150.O._10N.222.46.A6       | "Pontosvirinae"    | "Nahantvirus"   | [63]      |
| Vibrio phage 1.152.O._10N.222.46.E1       | "Pontosvirinae"    | "Nahantvirus"   |           |
| Vibrio phage 1.169.O._10N.261.52.B1       | "Pontosvirinae"    | "Nahantvirus"   |           |
| Vibrio phage 1.188.A._10N.286.51.A6       | "Pontosvirinae"    | "Nahantvirus"   |           |
| Vibrio phage 1.224.A._10N.261.48.B1       | "Pontosvirinae"    | "Mukerjeevirus" |           |
| Vibrio phage 1.261.O._10N.286.51.A7       | "Pontosvirinae"    | "Mukerjeevirus" |           |
| Vibrio phage vB_VspP_SBP1                 | "Pontosvirinae"    | "Nahantvirus"   | [60]      |

Vibrio phage JA–1                      | "Pontosvirinae"    | "Pacinivirus"    | [61]      |
Vibrio phage JSF3                       | "Pontosvirinae"    | "Pacinivirus"    | [62]      |
Vibrio phage phi 1                      | "Pontosvirinae"    | "Pacinivirus"    | [63]      |
Vibrio phage phi50–12                   | "Pontosvirinae"    | "Galateavirina"  | [64]      |
Vibrio phage pVco–5                     | "Pontosvirinae"    | "Stoningtovirus" |           |
Vibrio phage VBP32                      | "Pontosvirinae"    | "Stoningtovirus" |           |
Vibrio phage VBP47                      | "Pontosvirinae"    | "Pacinivirus"    | [60]      |
Vibrio phage VCO139                     | "Pontosvirinae"    | "Pacinivirus"    |           |
Vibrio virus vB_VspP_SBPI               | "Pontosvirinae"    | "Pacinivirus"    |           |

Table 1. Cont.
Table 1. Cont.

| Phage                          | Subfamily | Genus               | Reference |
|-------------------------------|-----------|---------------------|-----------|
| Xanthomonas phage RiverRider   |           | “Riverridervirus”   | [65]      |
| From metagenomes              |           |                     |           |
| Podoviridae sp. isolate ctda_1|           |                     |           |
| Podoviridae sp. ctLUJ1         |           |                     |           |
| Siphoviridae sp. isolate 355   |           | “Enquatrovirinae”   | Gamaleyavirus |
Figure 3. VCon\textsc{TACT2} network analysis. Members of the proposed “Schitoviridae” family are marked in red.

Panproteome analysis revealed that seventeen N4-like proteins are conserved in this proposed family of phages: RNAP 1 (EPNV4\_gp15), RNAP 2 (EPNV4\_gp16), vRNAP (EPNV4\_gp50), EPNV4\_gp24, EPNV4\_gp25, DNA polymerase (EPNV4\_gp39), EPNV4\_gp42, DNA primase (EPNV4\_gp43), EPNV4\_gp44, EPNV4\_gp52, EPNV4\_gp54, major capsid protein (EPNV4\_gp56), tape measure protein (EPNV4\_gp57), portal protein (EPNV4\_gp59), EPNV4\_gp67, large terminase subunit (EPNV4\_gp68), and EPNV4\_gp69 (Table 2).

Table 2. Panproteome analysis of N4-like viruses using three different approaches.

| # | N4\_Product | N4 Locus Tag | N4 Protein Accession | PIRATE | Proteinortho\_30 | CoreGenes 5.0 |
|---|-------------|--------------|----------------------|--------|------------------|---------------|
| 1 | RNAP 1      | EPNV4\_gp15  | YP\_950493.1         | Y      | Y                | Y             |
| 2 | RNAP 2      | EPNV4\_gp16  | YP\_950494.1         | N      | Y                | Y             |
| 3 | AAA+ ATPase | EPNV4\_gp24  | YP\_950502.1         | *      | Y                | Y             |
| 4 | gp25        | EPNV4\_gp25  | YP\_950503.1         | N      | N                | Y             |
| 5 | DNA polymerase | EPNV4\_gp39 | YP\_950517.1         | Y      | Y                | Y             |
| 6 | gp42        | EPNV4\_gp42  | YP\_950520.1         | Y      | Y                | Y             |
| 7 | DNA primase | EPNV4\_gp43  | YP\_950521.1         | Y      | Y                | Y             |
| 8 | gp44        | EPNV4\_gp44  | YP\_950522.1         | Y      | Y                | Y             |
| 9 | vRNAP       | EPNV4\_gp50  | YP\_950528.1         | N      | N                | Y             |
| 10 | 16.5 kDa protein | EPNV4\_gp52 | YP\_950530.1         | Y      | Y                | Y             |
| 11 | gp54        | EPNV4\_gp54  | YP\_950532.1         | N      | N                | Y             |
| 12 | Major capsid protein | EPNV4\_gp56 | YP\_950534.1         | Y      | Y                | Y             |
| 13 | gp57 (tape measure) | EPNV4\_gp57 | YP\_950535.1         | N      | *                | Y             |
| 14 | 94 kDa protein (portal vertex protein) | EPNV4\_gp59 | YP\_950537.1         | Y      | Y                | Y             |
| 15 | 30 kDa protein Terminate, large subunit | EPNV4\_gp67 | YP\_950545.1         | N      | N                | Y             |
| 16 | gp68        | EPNV4\_gp68  | YP\_950546.1         | Y      | Y                | Y             |
| 17 | gp69        | EPNV4\_gp69  | YP\_950547.1         | Y      | N                | Y             |

* Indicates presence in 113/114 genomes.
Based on the different analyses, we propose a new family "Schitoviridae" in honor of Gian Carlo Schito who isolated Escherichia phage N4, the first isolated species of this group.

2.3. Proposal of New Subfamilies and Genera

Results of an all-by-all pairwise nucleotide identity analysis or intergenomic similarity analysis with VIRIDIC gave strong evidence for the proposal of eight new subfamilies and 30 genera which were confirmed by phylogenetic analysis of the terminase large subunit and vRNA polymerase genes, i.e., all proposed taxa are monophyletic in these marker gene trees (Supplementary Figures S1 and S2). In line with previously established taxa, we used 95% and 70% nucleotide sequence identity over the length of the genome as species and genus demarcation criteria, respectively [11,12,68,69]. At the subfamily level, members of the same subfamily share at least 40% intergenomic distance as calculated with VIRIDIC, with members of different subfamilies sharing little to no nucleotide identity [70].

The proposed subfamily “Migulavirinae” consists of two previously ratified genera (eight species), Litunavirus and Luzseptimavirus, representing phages with Pseudomonas aeruginosa as their host. The subfamily “Enquatrovirinae” contains three genera (14 species), Gamaleyavirus, Enquatrovirus and the newly proposed genus “Kaypoctavirus” and includes phages infecting members of the Enterobacteriaceae like E. coli, Shigella boydii or Klebsiella pneumoniae. N4-like viruses infecting Achromobacter xylosoxidans were grouped into four genera (eight species) in the proposed subfamily “Rothmandenesvirinae” in honour of Lucia Rothman-Denes, who worked on N4 and its RNA polymerases. The subfamily “Erskinevirinae” was named after John M. Erskine who in the early 1970s was one of the first people to isolate phages against Erwinia. It consists of two genera, “Yonginvirus” and Johnsonvirus, with three species and represents most of the N4-like viruses against Erwinia. The relatively large subfamily “Rhodovirinae” consists of seven genera, “Aorunvirus”, “Raunefjordvirus”, “Aoquinvirus”, “Pomeroyivirus”, “Sanyabayvirus”, “Plymouthvirus” and Baltimorevirus, and contains aquatic viruses infecting members of the Rhodobacteraceae. Two further proposed subfamilies (five genera), “Fuhrmanvirinae” (named after American oceanographer and marine biologist Jed Alan Fuhrman) and “Pontosvirinae”, mainly consist of phages against marine Vibrio species. The “Humphriesvirinae” subfamily in honour of James C. Humphries (1914–1992), who was the first to isolate a Klebsiella phage, comprises five genera with viruses infecting different genera of the Enterobacteriaceae like Escherichia, Klebsiella or Salmonella.

3. Discussion

The constantly rising number of sequences provides the scientific community with valuable data to work with to answer various scientific questions. However, the taxonomic classification of phage genomes has not kept pace which has led to the presence of large numbers of unclassified genomes in the INSDC. While the ICTV makes a huge effort to manage this problem and improvements have been made on the genus and subfamily level (2019: 103 proposals, 2020: 188 proposals submitted [68]), it is clear that at the family level that concerted efforts, both by the ICTV and the wider community of phage biologists are required to address the issue of family-level classification. The creation of the family Herelleviridae from the subfamily Spounavirinae and related phages [69], provided the blueprint for the creation of new families of tailed phages, and the start to the dismantling of the morphology-based families Myoviridae, Siphoviridae and Podoviridae. Following from that example, we used some of the methods trialed and tested for the creation of a new family (Phage Proteomic Tree, vConTACT2) and the delineation of its internal structure (genome-distance comparisons, phylogenetic analysis of signature genes) to define the new family “Schitoviridae” of N4-like phages, to be removed from the family Podoviridae.
4. Materials and Methods

4.1. vConTACT2 Analysis

To create the gene-sharing network, a total of 16,050 phage contigs ([http://millardlab.org/](http://millardlab.org/)) [71] were reannotated using Prodigal v2.6.3 and clustered using vConTACT2.0 [72] and the ProkaryoticViralRefSeq database v94. The resulting network was visualised and annotated using Cytoscape v3.8.0.

4.2. Panproteome Analysis

To identify conserved proteins present in bacteriophages comprising the proposed family, all genomes were reannotated using Prokka v1.14.5 [73] and predicted CDS mapped against the VOG hmm database using hmmscan. GFF3 files or protein FASTA files were used as input for Proteinortho v6 [74] and PIRATE v1.0.4 [75], respectively.

For panproteome construction with PIRATE the settings used were 30 and 35% identity threshold, cdhit lowest percentage id of 95 and e-value for BLAST hit filtering of 1E-5. For Proteinortho, the search options were adjusted so that the minimum percent identity and coverage of the best blast hits were 30% and 50%, respectively. All other parameters were left as default.

The CoreGenes5.0 webserver (https://coregenes.ngrok.io/) was used with the OrthoMCL option with E-value of 1e-5. CoreGenes5.0 uses the GET_HOMOLOGUES package to implement the ortholog clustering [76,77]. We considered signature genes to be gene products present in all members of the proposed family where there was consensus between two or more of the analyses.

4.3. VIRIDIC Analysis

The Bacterial and Archaeal Viruses Subcommittee uses nucleotide based sequence similarities as a crucial feature for taxonomic classification of viruses at the ranks of species and genus. We therefore employed the online tool VIRIDIC (Virus Intergenomic Distance Calculator, [http://rhea.icbm.uni-oldenburg.de/VIRIDIC/](http://rhea.icbm.uni-oldenburg.de/VIRIDIC/)) [70] for the calculation of pairwise intergenomic similarities amongst the phage genomes of this study. We have chosen 95% DNA sequence identity as the criterion for demarcation of species in genera. Each of the proposed species differs from the others with more than 5% at the DNA level. For the demarcation of genera and subfamilies, we have chosen 70% and 40% DNA sequence identity, respectively. Based on this analysis, new genera and subfamilies were identified (Supplementary Table S1).

Supplementary Materials: The following are available online at [http://www.mdpi.com/2079-6382/9/10/663/s1](http://www.mdpi.com/2079-6382/9/10/663/s1).

Figure S1: Phylogenetic analysis using the terminase protein sequences of N4-like phages, respectively. The amino acid sequences were compared using MUSCLE with MEGA7 [78]. The tree was constructed using the maximum likelihood algorithm. The percentages of replicate trees were assessed with the bootstrap test (100). Figure S2: Phylogenetic analysis using the vRNA polymerase protein sequences of N4-like phages, respectively. The amino acid sequences were compared using MUSCLE with MEGA7 [78]. The tree was constructed using the maximum likelihood algorithm. The percentages of replicate trees were assessed with the bootstrap test (100). Figure S3: ViPTree analysis of N4-like viruses with related podoviruses. Table S1: VIRIDIC analysis of N4-like phages.

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Conflicts of Interest: With the exception of P.M. all authors are members of the Bacterial and Archaeal Viruses Subcommittee of the International Committee on Taxonomy of Viruses (ICTV).
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