Discovery of three cycloviruses in fecal samples from silver-haired bats (Lasionycteris noctivagans) in Arizona (USA)

Ciara Harding1,2 · Brendan B. Larsen3,4 · Sophie Gryseels3,5,6 · Hans W. Otto3 · Crystal Suazo1,2 · Simona Kraberger1 · Nathan S. Upham2 · Michael Worobey3 · Koenraad Van Doorslaer7 · Arvind Varsani1,2,8

Received: 21 March 2022 / Accepted: 3 August 2022 / Published online: 31 August 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2022, corrected publication 2022

Abstract

Bats harbour a diverse array of viruses, some of which are zoonotic, and are one of the most speciose groups of mammals on earth. As part of an ongoing bat-associated viral diversity research project, we identified three cycloviruses (family Circoviridae) in fecal samples of silver-haired bats (Lasionycteris noctivagans) caught in Cave Creek Canyon of Arizona (USA). Two of the three identified genomes represent two new species in the genus Cyclovirus. Cycloviruses have been found in a wide range of environments and hosts; however, little is known about their biology. These new genomes of cycloviruses are the first from silver-haired bats, adding to the broader knowledge of cyclovirus diversity. With continuing studies, it is likely that additional viruses of the family Circoviridae will be identified in Arizona bat populations.

Keywords Circoviridae · Cyclovirus · Lasionycteris noctivagans

GenBank accession nos. OM262453 · OM262454 · OM262459

Bats (order Chiroptera) are one of the most abundant and diverse groups of mammals, with 1,448 extant species currently recognized [1, 2]. Bats are associated with an abundant virome and have primarily been studied from the perspective of zoonotic transmission, with an emphasis on coronavirus, filoviruses, paramyxoviruses, and rhabdoviruses [3–5]. Over the last decade, there has been a significant interest in analyzing bat-associated viruses using viral metagenomic approaches, which has resulted in the identification of numerous novel and known DNA and RNA viruses [4–6]. However, limited discovery work has been done on bat-associated viruses in Arizona [7–9], even through southern Arizona harbors the greatest diversity of bats of any comparably sized region in the United States of America [2].

Our field research was conducted in Madrean evergreen-woodland on Cave Creek in the Chiricahua Mountains (Cochine County, Arizona). To capture bats, we deployed mist nets (2.6, 4, and 6 m; Avinet Inc., Portland, ME) across areas of calm water on sections of Cave Creek where the
In this report, we focus on the three circovirus-like contigs for which, based on the de novo-assembled contigs, we designed abutting primer pairs (Table 1) in order to recover these full genomes. The RCA product was used as a template with the specific primer pairs (Table 1) to amplify the full genomes of the three circovirus-like sequences using Kapa HiFi DNA polymerase (Roche Diagnostics, USA) according to the manufacturer’s recommendations. The amplions were resolved by electrophoresis in a 0.7% agarose gel, purified, and cloned into the vector pJET 1.2 (Thermo Fisher Scientific, USA). Competent XL1-Blue Escherichia coli cells were transformed with the recombinant plasmids, which were then sequenced by the Sanger method at Macrogen Inc. (South Korea), using primer walking. The Sanger sequences were assembled using Geneious Prime 2021.0.3 (Biomatters Ltd., New Zealand). Open reading frames (ORFs) were identified using ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/).

A BLASTn web search against the NCBI nt database of the three complete Sanger-sequenced genomes (1758–2320 nt; OM262453, OM262454, OM262459) revealed that they shared the highest similarity with various cycloviruses of the family Circoviridae. Circoviridae is a family of single-stranded circular DNA viruses with an ambisense genome organization and two ORFs coding for the capsid protein (Cp) and replication-associated protein (Rep) [16]. There are two genera within this family, Circovirus and Cyclovirus [16]. Unlike members of the genus Circovirus that have been implicated in various diseases in mammals and birds (e.g., postweaning multisystemic wasting syndrome in pigs and psittacine beak and feather disease in parrots), relatively little is known about members of the genus Cyclovirus [17]. A feature that distinguishes the genomes of circoviruses and

### Table 1  Summary of the primers used to recover the complete genomes of the three cycloviruses, with their GenBank accession numbers and genome lengths as well as their nonanucleotide, HUH endonuclease, and superfamily 3 helicase motifs

| Virus       | Primer pair                          | Accession no. | Genome length (nt) | Nonanucleotide | Motif I | Motif II | Motif III | Walker A | Walker B | Walker C |
|-------------|--------------------------------------|---------------|--------------------|----------------|---------|---------|-----------|----------|----------|----------|
| Chifec virus UA15_35 | F: AGATCGTGTTCACT-GCCATCGTATTAGTT R: TAAAGCAAGACTGGT-GACACTGTCGTT | OM262453      | 1758               | AAG-TATTAC     | CFTKNN | RHLQGY | QNLTYCSK | GPP-GTGC | IIDDF    | FISSN    |
| Chifec virus UA15_517 | F: TTGTCAATCTGTCG-GTATTGATTAGAG R: ATTTGAGGTTATG-TAAACACAGCATCGA | OM262454      | 1779               | TAG-TATTAC     | VYTLNN | PHLQGF | DNQKYCSK | GEP-GTGC | IIDDF    | WITSN    |
| Chifec virus UA15_2320 | F: GAGAGGTGTTTCAGA-CAATAGTCCCTTTT R: AGTTTGAAAGATTTG-GAACCTTACCAAGTC | OM262459      | 2320               | TAGTAT-TAC     | CWTKNN | RHLQCY | QNKDYSK | GPTRT    | VLDDYIITSN | LAA      |

(n = 3; 1119–7008 nt), and Siphoviridae (n = 51; 767–3265 nt).
Discovery of three cycloviruses in fecal samples from silver-haired bats (Lasionycteris noctivagans) in Arizona…

Fig. 1 Maximum-likelihood phylogenetic tree inferred from the alignment of the genome sequences of cycloviruses and rooted with the reverse compleлемент sequences of members of the genus Circovirus. The source at the level of order (Anseriformes, Araneae, Artiodactyla, Blattodea, Carnivora, Chiroptera, Diptera, Eulipotyphla, Galliformes, Hymenoptera, Odonata, Passeriformes, Perissodactyla, Primates, Rodentia and Fabales) of the genomes is shown in color-coded boxes. Branches with more members within a species have been collapsed. Accession numbers are provided for unclassified cycloviruses and for species that only has a single member. The phylogenetic tree was rooted with reverse complement genome sequences of representative members of the genus Circovirus.
cycloviruses is the orientation of the rep and cp genes relative to the conserved nonanucleotide motif. The rep gene is on the virion sense strand, whereas the cp gene is on the complementary strand for members of the genus Circovirus and vice versa for those of the genus Cyclovirus [17]. Although cyclovirus genomes have been identified in various environmental and animal samples [17], including those obtained from members of the orders Anseriformes (n = 1), Aranaeae (n = 2), Artiodactyla (n = 7), Blattodea (n = 1), Carnivora (n = 52), Chiroptera (n = 29), Diptera (n = 1), Eulitophtyla (n = 7), Galliformes (n = 11), Hymenoptera (n = 3), Odonata (n = 35), Passeriformes (n = 10), Perissodactyla (n = 1), Primates (n = 27), Rodentia (n = 7), and from a plant of the order Fabales (n = 1), no definite hosts have been identified so far, and thus, their biology is unknown.

In the three cyclovirus genomes from silver-haired bats, we identified a conserved nonanucleotide motif, “NAG-TATTAC”. Additionally, in the Rep sequence, we identified RCR endonuclease and superfamily 3 (SF3) helicase motifs (Table 1). To determine the phylogenetic relationship of the viruses to other cycloviruses, 195 genome sequences of cycloviruses were downloaded from the GenBank database on 10 Jan 2022 and aligned with those from silver-haired bats identified in this study. Reverse complement genome sequences of two circoviruses (porcine circovirus 1 and 2) were used as an outgroup. These were aligned using MAFFT v7.113 in AUTO mode [18], and the resulting alignment was used to infer a maximum-likelihood phylogenetic tree using PhyML 3.0 [19] with the GTR + I + G substitution model. Branches with less than 60% bootstrap support were collapsed using TreeGraph2 [20]. The phylogenetic tree was visualized and edited in iTOL v6 [21].

The three cyclovirus genomes from silver-haired bats share ~57–60% genome-wide pairwise identity (calculated using SDT v1.2 [22]) and are phylogenetically distinct from each other (Fig. 1; Supplementary Data 1). Chifec virus UA15_35 (OM262454) shares 87% genome-wide pairwise identity with dragonfly cyclovirus 6 (KC512918), which is the sole known member of the species Dragnfly associated cyclovirus 6 (Supplementary Data 1). Members of the family Circoviridae are classified into species based on their genome-wide pairwise identity with a species demarcation threshold of 80% [17]; thus, chifec virus UA15_35 may be a member of the species Dragnfly associated cyclovirus 6. Chifec virus UA15_35 (OM262453) shares <61% identity with all other cycloviruses and clusters phylogenetically with members of the species Horse associated cyclovirus 1 (Fig. 1; Supplementary Data 1). Chifec virus UA15_2320 (OM262459) shares <70% identity with all other cycloviruses and clusters phylogenetically with Caesalpinia ferrea associated virus (MT707947) and members of the species Bat associated cyclovirus 7 and Mouse associated cyclovirus 1 (Fig. 1; Supplementary Data 1). Chifec virus UA15_35 and UA15_2320, based on the species demarcation threshold, each represent a new species of cycloviruses.

The three cycloviruses identified in the fecal samples of silver-haired bats are the first from this bat species, and only two other cycloviruses from bats have been described in the USA, one from the pallid bat (Antrozous pallidus) [23] and one from the Mexican free-tailed bat (Tadarida brasiliensis) [24]. Besides these, other cycloviruses have been identified in bat guano and tissue from bats of various species (Chalinolobus gouldii, Molossus molossus, Myotis spp., Nyctalus noctula, Pipistrellus nathusii, Plecotus auritus, Pteropus tonganus, Rhinolophus ferrumequinn, Rhinolophus pusillus, Tadarida brasiliensis, Tylonycteris pachypus, Vespertilio superans) from Australia, Brazil, China, Hungary, Tonga, and Ukraine [12, 25–28]. Since we detected the cycloviruses in bat feces, we are unable to determine whether these are diet-related viruses or ones that truly infect silver-haired bats. Examination of feces across their range has shown that silver-haired bats feed on a variety of insects, including representatives of the orders Lepidoptera, Hemiptera, Coleoptera, Diptera, and Trichoptera [10, 11]. Thus, it is likely that the cycloviruses identified in silver-haired bat feces infect these insects. Nonetheless, given the increased focus on bats as reservoirs of various viruses, it is likely that the identification of novel cycloviruses in bat samples will continue.
Discovery of three cycloviruses in fecal samples from silver-haired bats (Lasionycteris noctivagans) in Arizona…

References

1. Burgin CJ, Colella JP, Kahn PL, Upham NS (2018) How many species of mammals are there? J Mammal 99:1–14
2. MMM (2022) Mammal Diversity Database v1.8. Zenodo https://doi.org/10.5281/zenodo.4139818
3. Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T (2006) Bats: Important Reservoir Hosts of Emerging Viruses. Clin Microbiol Rev 19:531–545
4. Letko M, Seifert SN, Olival KJ, Plovrigh RK, Munster VJ (2020) Bat-borne virus disease, spillover and emergence. Nat Rev Microbiol 18:461–471
5. Van Brussel K, Holmes EC (2022) Zoonotic disease and virome diversity in bats. Curr Opin Virol 52:192–202
6. Tan CW, Yang X, Anderson DE, Wang LF (2021) Bat virome research: the past, the present and the future. Curr Opin Virol 49:68–80
7. Kuzmin IV, Shi M, Orciari LA, Yager PA, Velasco-Villa A, Kuzmina NA, Streicker DG, Bergman DL, Rupprecht CE (2012) Molecular inferences suggest multiple host shifts of rabies viruses from bats to mesocarnivores in Arizona during 2001–2009. PLoS Pathog 8:e1002786
8. Larsen BB, Gryseels S, Otto HW, Worobey M (2022) Evolution and Diversity of Bat and Rodent Paramyxoviruses from North America. J Virol 96:e0109821
9. King KM, Larsen BB, Gryseels S, Richet C, Kraberger S, Jackson R, Worobey M, Harrison JS, Varsani A, Doorslaer KV (2022) Co-evolutionary analysis implicates TLR9 in the restriction of papillomavirus infection. mBio 13:e0005422
10. Kunz TH (1982) Lasionycteris noctivagans. Mammalian Species:1–5
11. Jones JK (1973) Notes on the distribution and natural history of bats in southeastern Montana. Museum, Texas Tech University
12. Male MF, Kraberger S, Stainton D, Kav I, Varsani A (2016) Cycloviruses, gemycircularviruses and other novel replication-associated protein encoding circular viruses in Pacific Flying fox (Pteropus tonganus) faeces. Infect Genet Evol 39:277–292
13. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120
14. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prijibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesluk G, Alekseyev MA, Pevzner PA (2012) SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. J Comput Biol 19:455–477
15. Altshul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410
16. Breitbart M, Delwart E, Rosario K, Segales J, Varsani A, Ictv Report C (2017) ICTV Virus Taxonomy Profile: Circoviridae. J Gen Virol 98:1997–1998
17. Rosario K, Breitbart M, Harrach B, Segales J, Delwart E, Bagnini P, Varsani A (2017) Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. Arch Virol 162:1447–1463
18. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780
19. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321
20. Stower BC, Muller KF (2010) TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics 11:7
21. Letunic I, Bork P (2019) Interactive Tree Of Life (iTOl) v4: recent updates and new developments. Nucleic Acids Res 47:W256–W259
22. Muhire BM, Varsani A, Martin DP (2014) SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS ONE 9:e108277
23. Li L, Victoria JG, Wang C, Jones M, Fellers GM, Kunz TH, Delwart E (2010) Bat guano virome: predominance of dietary viruses from insects and plants plus novel mammalian viruses. J Virol 84:6955–6965
24. Li L, Shan T, Soji OB, Alam MM, Kunz TH, Zaidi SZ, Delwart E (2011) Possible cross-species transmission of circoviruses and cycloviruses among farm animals. J Gen Virol 92:768–772
25. Ge X, Li J, Peng C, Wu L, Yang X, Wu Y, Zhang Y, Shi Z (2011) Genetic diversity of novel circular ssDNA viruses in bats in China. J Gen Virol 92:2646–2653
26. Kemenesi G, Kurucz K, Zana B, Foldes F, Urban P, Vlaschenko A, Kravchenko K, Budinski I, Szodoray-Paradi F, Bucs S, Cere J, Czos I, Szodoray-Paradi A, Estok P, Gorfol T, Boldogh S, Jakab F (2018) Diverse replication-associated protein encoding circular DNA viruses in guano samples of Central-Eastern European bats. Arch Virol 163:671–678
27. Lima FE, Cibulski SP, Dos Santos HF, Teixeira TF, Varel AP, Roehe PM, Delwart E, Franco AC (2015) Genomic characterization of novel circular ssDNA viruses from insectivorous bats in Southern Brazil. PLoS ONE 10:e0118070
28. Wu Z, Yang L, Ren X, He G, Zhang J, Yang J, Qian Z, Dong J, Sun L, Zhu D, Yu J, Yang F, Zhang S, Jin Q (2016) Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. ISME J 10:609–620

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under applicable law. This article, or parts thereof, may not be further reproduced, stored or transmitted to any retrieval system, nor may it be transmitted in any form or by any means—electronic, photocopy or otherwise—without the written consent of the Publisher.

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under applicable law. This article, or parts thereof, may not be further reproduced, stored or transmitted to any retrieval system, nor may it be transmitted in any form or by any means—electronic, photocopy or otherwise—without the written consent of the Publisher.

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under applicable law. This article, or parts thereof, may not be further reproduced, stored or transmitted to any retrieval system, nor may it be transmitted in any form or by any means—electronic, photocopy or otherwise—without the written consent of the Publisher.