UNIVERSITY OF PÉCS
NATIONAL LABORATORY
OF VIROLOGY

Universal, amplicon-based sequencing method for *Canine Morbillivirus* (CDV)

Gábor Endre Tóth[1,2], Zsófia Lanszki[1,2], Gábor Kemenesi[1,2]
[1] University of Pécs
[2] National Laboratory of Virology (Hungary)
E-mail: toth.gabor.endre@gmail.comA

Introduction

Nanopore sequencing technology provide the only portable and low-cost solution to generate viral genome information without limitation in fragment length. Amplicon based sequencing methods proved their relevance during human epidemics (Ebola[1], Dengue[2], Zika virus[1]) and the current pandemic (SARS-CoV 2). Th Canine distemper virus (CDV) is a highly contagious virus that causes multi-systemic, sub-clinical to fatal diseases in a wide range of carnivore species and affecting domestic and wild animals worldwide[3]. CDV is highly prone to cross-species transmission between domestic and wildlife reservoir hosts, representing a significant OneHealth challenge on the wildlife-domestic animals interface[4,5]. The viral genome is ~ 15 kb and encodes six structural proteins and classified into 18 major lineages. Hitherto only 195 almost full genomes available in the NCBI database (27.04.2022.). Genomic data is essential to design efficient diagnostic tests or vaccines. Without these the effective countermeasures are not available. As CDV affect multiple host species there is huge lack of understanding in the natural circulation and can have impact on the conservation of endangered species beyond the animal health issues. Our main goal was to develop an universal amplicon-based sequencing method that is able to sequence all known CDV lineage.

Materials and methods

Collection of high quality CDV genomes
Length: >14 000 base

Multiple sequence alignment
MUSCLE, MAFFT

Primer design with PrimalScheme[1]
1000 and 2000 basepair set
113 and 59 primers

Collecting CDV positive samples
***Vulpes vulpes* (19), *Lutra Lutra* (1), *Mustela eversmanii* (1), *Mustela putorius* (1), *Martes foina* (2)**

Reverse Transcription and Multiplex PCR
Superscript IV (Invitrogen, USA)
Q5 Hot Start HF Polymerase (New England Biolabs, USA)

NGS Library Preaparation (End-Prep, Adapter and barcode ligation)
NEBNext Ultra II End Repair/dA-Tailing Module (NEB, USA)
EXP-NBD196 and LSK-109 (Oxford Nanopore Technologies, UK)

Nanopore sequencing
R9.4.1 (FLO-MIN106D) flow cell

Basecalling and Demultiplexing
Guppy (version 5.0.7.) with dna_r9.4.1_450bps_sup model

Mapping and Polishing
Genious mapper (version Geneious Prime 2021.2.2)
Medaka (version 1.4.2)

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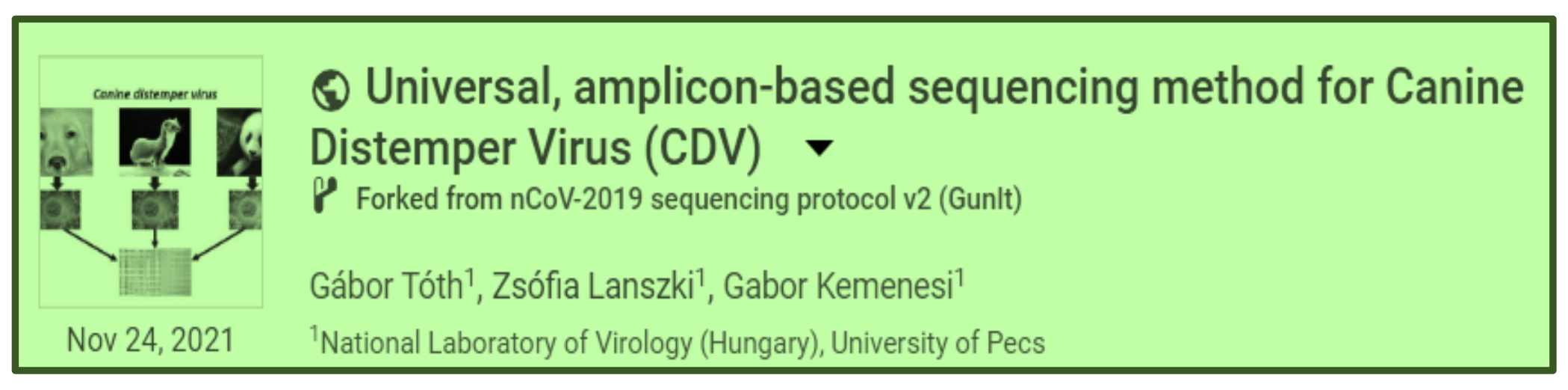
Maximum Likelihood phylogeny of complete CDV genomes
MEGA (version 11.0.11)

The detailed protocol is available at our laboratory protocols.io page under the link: https://www.protocols.io/view/universal-amplicon-based-sequencing-method-for-can-x54v9j6mpg3e/v1


Universal, amplicon-based sequencing method for Canine Distemper Virus (CDV)
Forked from nCoV-2019 sequencing protocol v2 (GunIt)
Gábor Tóth[1], Zsófia Lanszki[1], Gabor Kemenesi[1]
[1]National Laboratory of Virology (Hungary), University of Pecs
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References
1 Quick, J. et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nat. Protoc.* **12**, 1261–1266 (2017).

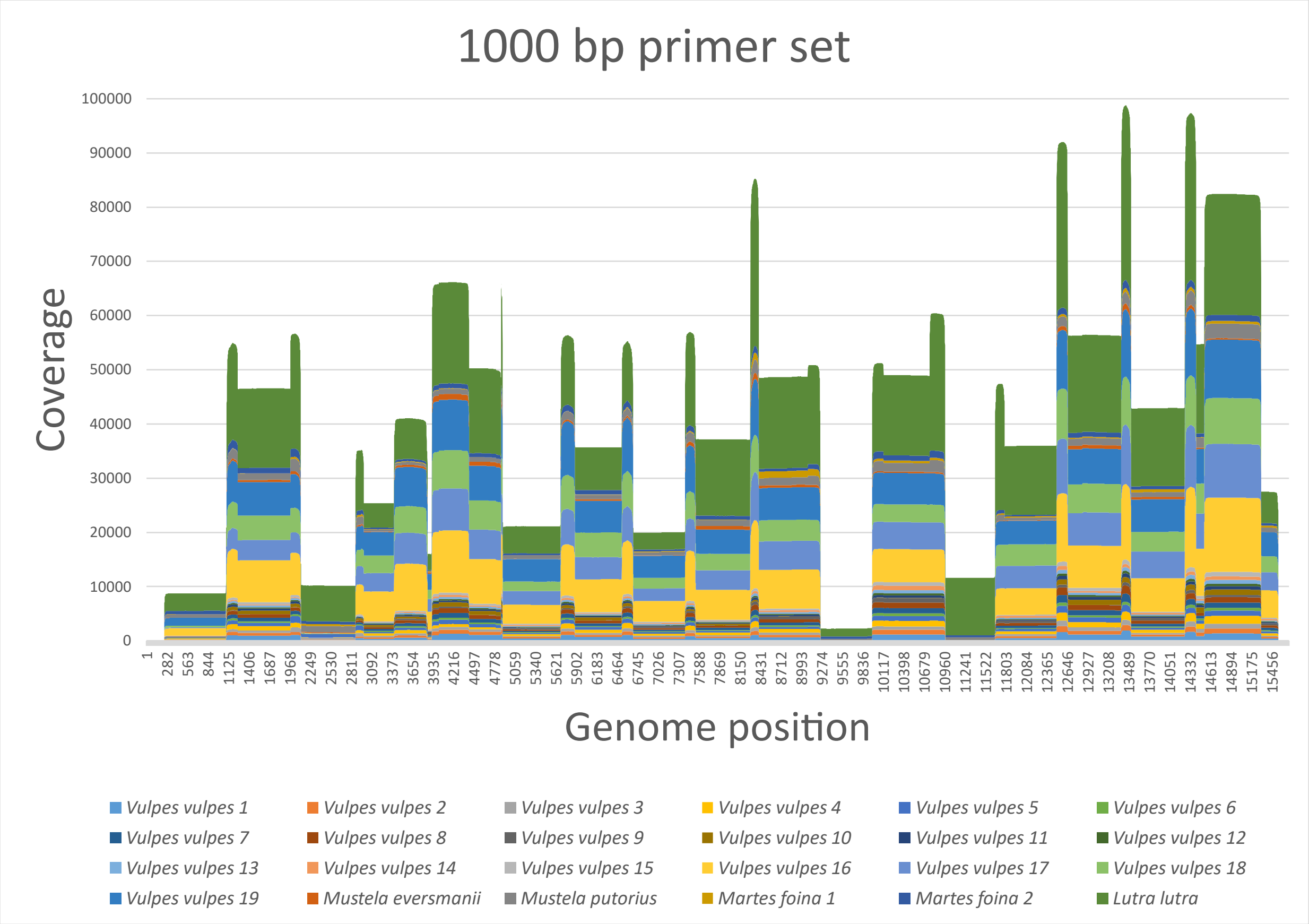
2 Stubbs, S.C.B., Blacklaws, B.A., Yohan, B. *et al.* Assessment of a multiplex PCR and Nanopore-based method for dengue virus sequencing in Indonesia. *Virol J* **17,** 24 (2020). https://doi.org/10.1186/s12985-020-1294-6

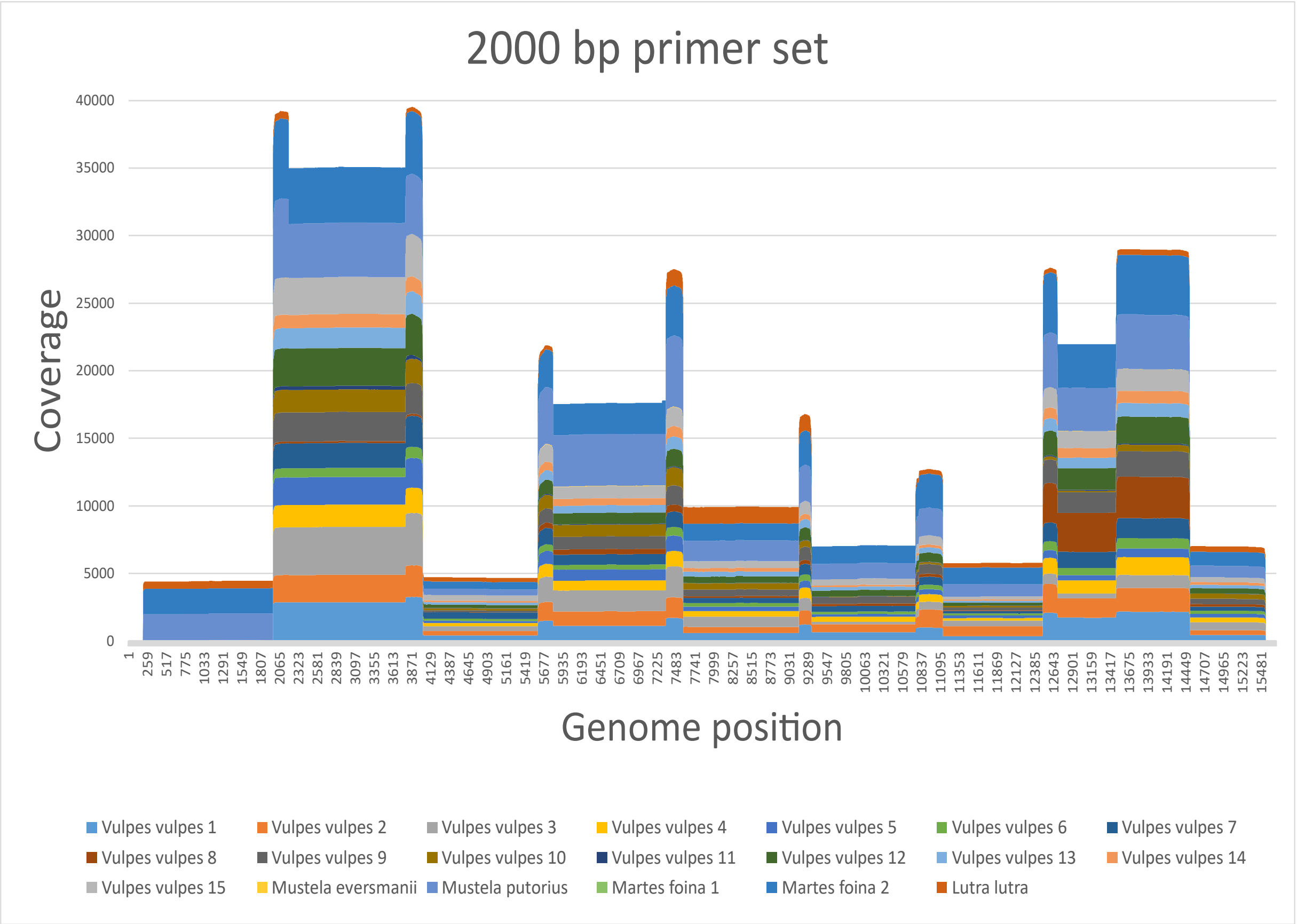
3 Martinez-Gutierrez, M. & Ruiz-Saenz, J. Diversity of susceptible hosts in canine distemper virus infection: A systematic review and data synthesis. *BMC Vet. Res.* **12**, 1–11 (2016).

4 McCarthy, A. J., Shaw, M.-A. & Goodman, S. J. Pathogen evolution and disease emergence in carnivores. *Proc. R. Soc. B Biol. Sci.* **274**, 3165–3174 (2007).

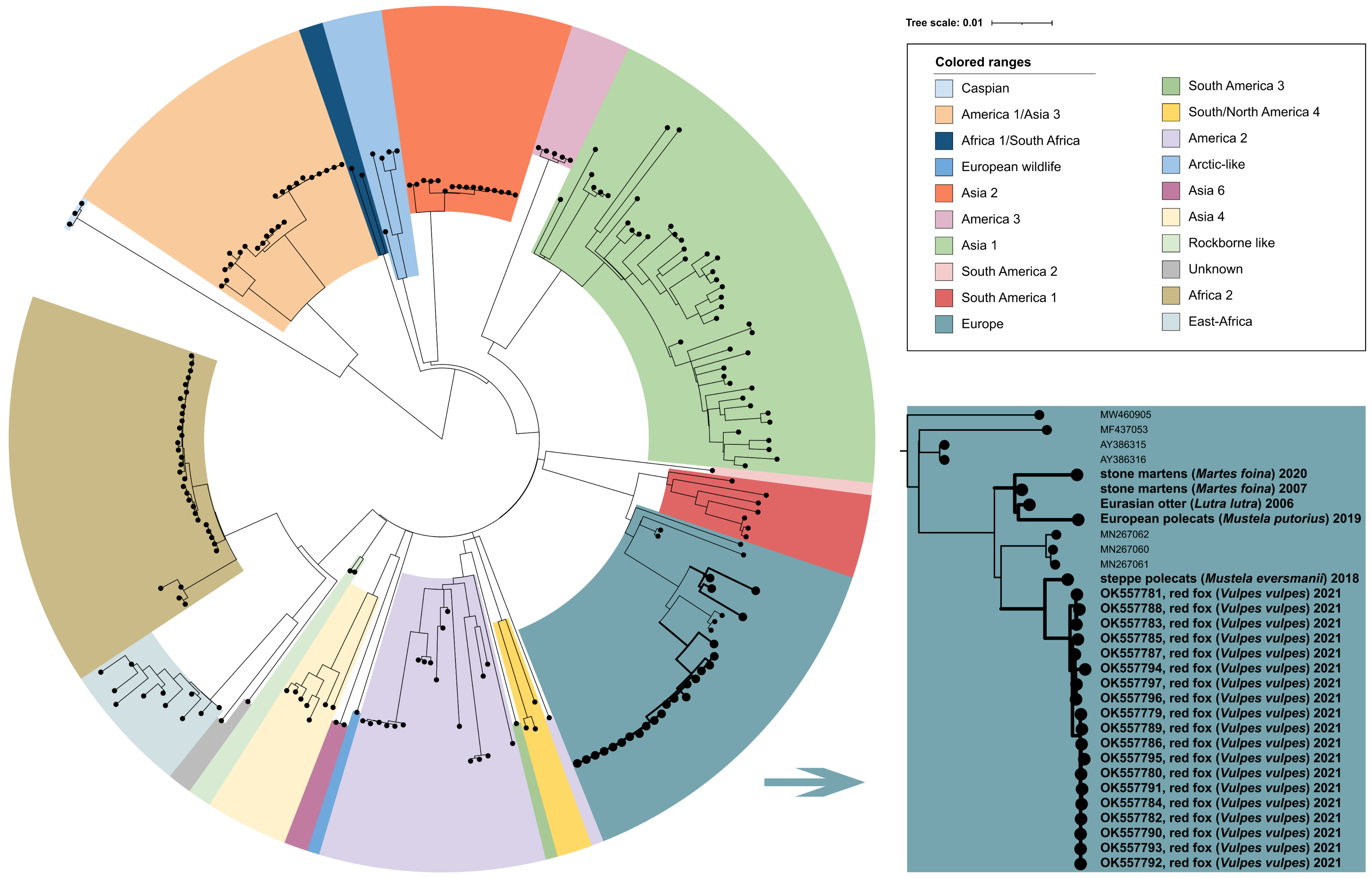
5 Ludlow, M., Rennick, L. J., Nambulli, S., de Swart, R. L. & Paul Duprex, W. Using the ferret model to study morbillivirus entry, spread, transmission and cross-species infection. *Curr. Opin. Virol.* **4**, 15–23 (2014).

Results





The graph shows the coverage of different samples with the designed 1000 and 2000 bp primer set.



This is a Maximum Likelihood phylogeny of CDV full genomes with GTR+G+I substitution model. The highlighted part is containing the generated novel sequences from different hosts.

Novel identified hosts for CDV:


Steppe polecat
(Mustela eversmanii)


Eurasian otter
(Lutra lutra)

Concluding Remarks

-We were able to generate 24 almost complete CDV genome which is 12,3% of the currently available data in the NCBI database.

-CDV can have importance in the conservation of endangered or threatened species

-This method was designed to be universal but it should be widely tested with multiple CDV lineages to evaluate it's efficiency.

Related publication

Lanszki Z, Tóth GE, Schütz É, Zeghbib S, Rusvai M, Jakab F, Kemenesi G. Complete genomic sequencing of canine distemper virus with nanopore technology during an epizootic event. Sci Rep. 2022 Mar 8;12(1):4116. doi: 10.1038/s41598-022-08183-3. PMID: 35260784; PMCID: PMC8904823.

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