Feline chaphamaparvovirus (FeChPV) in cats with enteritis and upper respiratory tract disease (URTD)

Short Running Title: FeChPVs in cats

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Summary

Feline chaphamaparvovirus (FeChPV) is a novel parvovirus, first discovered in a multi-facility feline shelter in Canada in 2019, during an outbreak of acute gastro-enteritis (AGE) in cats, and detected at high prevalence (47.0%) in faecal samples. Whether this finding was anecdotical or similar viruses are common components of feline virome is still unclear. Also, the potential impact of this virus on feline health is uncertain. Herewith, a case-control study was performed to investigate whether this novel parvovirus may play a role as enteric pathogen, screening samples collected from cats with and without AGE signs. Furthermore, we extended the research by testing archival paired oropharyngeal and ocular samples collected from cats with or without upper respiratory tract disease (URTD). FeChPV DNA was detected at high prevalence rate (36.8%) in clinical cases, representing the most frequently identified enteric virus, followed by feline panleukopenia parvovirus (FPV) (23.7%), feline enteric coronavirus (FECV) (5.3%), feline kobuvirus (FeKoV) (5.3%) and noroviruses (NoV) (5.3%). The different prevalence rates of FeChPV between the case and control group were statistically significant, suggesting a possible association of the virus with acute gastro-enteric disease. The virus was also detected at low rate in the respiratory samples of cats with (3.3%) or without URTD (4.3%), although there was no significant association between FeChPV and URTD. The complete VP encoding gene was determined for five viruses and the nearly full-length genome was reconstructed for three viruses, namely 313R/2019/ITA, 284R/2019/ITA and 49E/2019/ITA. In the NS1-based tree the Italian strains clustered tightly with the two FeChPV prototypes detected in Canada, within a monophyletic cluster related to but clearly distinct from canine chaphamaparvovirus, currently classified in the species *Carnivore chaphamaparvovirus 1*.

Keywords: parvoviruses, feline chaphamaparvoviruses, cats, enteritis, URTD

Introduction

Chaphamaparvoviruses (ChPVs) are small, nonenveloped, icosahedral viruses with a single-stranded linear DNA genome of ~4.0-4.5 kb in length classified in the family *Parvoviridae* (subfamily *Hamaparvovirinae*) within the genus *Chaphamaparvovirus*. As ChPVs are genetically more related to invertebrate-infecting parvoviruses than to other members of the subfamily *Parvovirinae*, an update of *Parvoviridae* taxonomy has been made recently, with the introduction of the novel subfamily *Hamaparvovirinae*, encompassing divergent densoviruses and vertebrate-infecting parvoviruses (Pénzes et al., 2020). ChPV was originally identified by metagenomic investigations in the oropharyngeal swab sample collected from a fruit bat (*Eidolon helvum*) in Ghana (Africa) (Backer et al., 2013). Since then, ChPV-like viruses have been described in several additional animal species (Pénzes, de Souza, Agbandje-McKenna, & Gifford, 2019), including dogs and cats (Fahsbender et al., 2019; Hu, Liu, Chen, & Ji, 2020; Ji et al., 2020; Li et al., 2020; Palombieri et al., 2020). The first identification of ChPVs in domestic carnivores was documented in Colorado (USA) in 2017 on next generation sequencing (NGS) of faecal samples collected from two dogs with haemorrhagic diarrhoea of unknown aetiology (Fahsbender et al., 2019). In subsequent studies, viruses genetically close to the American canine ChPV strains have been detected in both dog and cat faecal specimens (Hu et al., 2020; Ji et al., 2020; Palombieri et al., 2020). Based on the complete sequence of the non-structural (NS1) protein, all strains of canine and feline origin segregated into the newly established species *Carnivore chaphamaparvovirus 1* (CaChPV-1) with an overall amino acid (aa) identity of 98.6-99.8% (Pénzes et al., 2020). In 2019 a novel ChPV was identified at high prevalence rate (47.0%, 8/17) in faecal samples of cats during an outbreak of acute gastro-enteritis (AGE) in a multi-facility feline shelter in British Columbia (Canada) (Li et al., 2020). In the NS1 the feline ChPV strains detected in Canada resulted genetically distant from CaChPV-1 strains of feline and canine origin (76.0-77.0% aa identities), i.e. under the cut-off adopted by the International Committee on Taxonomy of Viruses (ICTV) for classification of parvoviruses into the same

species (cut-off > 85.0% aa identity in the NS1) (Pénzes et al., 2020). High divergence among different CaChPV strains was also observed in the predicted variable regions (VRs) of the VP capsid protein (Palombieri et al., 2020), with several aa changes located in the main sites involved in tissue tropism and receptor attachment of parvoviruses (Halder, Ng, & Agbandje-McKenna, 2012; Kailasan et al., 2015).

The discovery of new viruses in humans or animals must be interpreted with caution and epidemiological data collected from independent studies in other geographical areas are required to understand whether the findings are only anectodical or a newly discovered virus is a stable and common component of the host virome. Furthermore, the potential clinical impact of ChPVs on feline health and its possible role as enteric pathogen remain to be clarified (Li et al., 2020). Epidemiological studies conducted for CaChPV-1 so far have revealed a low prevalence in diarrhoeic (1.5% - 4.3%) and healthy dogs (0% - 1.6%) and no significant association with enteric disease (Fahsbender et al., 2019; Hu et al., 2020; Palombieri et al., 2020). Similar results were also obtained when testing cats with (2/171) and without signs of AGE (0/378) (Ji et al., 2020). In the last few years, animal ChPVs have been associated with a variety of clinical signs. The mouse kidney parvovirus (MKPV) (*Rodent chaphamaparvoviruses 1* species) was recognised as cause of inclusion body nephropathy (IBN) and kidney fibrosis in mice (Roediger et al., 2018; Ge et al., 2020; Lee et al., 2020). Association between ChPV infection and clinical signs was found in a dead peafowl suffering of enteritis and pneumonia (Liu et al., 2020). More recently, a newly discovered ChPV was detected in lung, liver and brain samples collected from bearded dragons showing respiratory or neurological symptoms (Chang et al., 2020).

In this study, we extended the research of feline ChPVs (FeChPVs) in a case-control study by testing cats with enteritis or upper respiratory tract diseases (URTDs). Also, the nearly full-length genome of three strains was determined adding data on the genetic features of these viruses.

Material and methods

Sampling

Molecular screening for FeChPVs was performed by screening biological samples (enteric and respiratory) collected from a total of 411 cats. More in details, enteric specimens (n = 38) were collected between December 2019 and August 2020 from household cats (mainly indoor) with signs of AGE admitted to the veterinary hospital of the Faculty of Veterinary Medicine, University of Teramo (Italy). Control animals (n = 51) were randomly recruited among healthy household cats during visits for routine vaccination (Collection A). Informed consent was obtained from all animal owners.

Paired archived samples (oropharyngeal and oral swabs) collected during 2014-2017 from a total of 322 juvenile household cats (aged 3 to 8 months) with URTD signs (n = 183) or clinically healthy animals (n = 139), was included in the study (Collection B). All respiratory samples were previously screened for diagnostic purpose for feline calicivirus (FCV), feline herpesvirus type 1 (FHV-1) and *Chlamydia felis* (*C. felis*) by nested RT-PCR and PCR (Marsilio, Di Martino, Decaro & Buonavoglia, 2005; Di Martino, Di Francesco, Meridiani & Marsilio, 2007).

Molecular screening for FeChPVs

Each sample was immersed in 1 mL of phosphate-buffered saline (0.15 M, pH 7.2) and then centrifuged at 3000 x g for 3 min. DNA and RNA were extracted individually from 200 µl of viral suspension by using the QIAamp Cador Pathogen Mini Kit (Qiagen S.p.A., Milan, Italy), following the manufacturer's instructions and stored at −80 °C until use. The presence of FeChPV DNA was assessed by heminested PCR using diagnostic primers targeting a 310-bp region of the NS1 protein encoding gene of the feline prototype strain ChPV-IDEXX-1 (GenBank accession number: MN396757) (Li et al., 2020). Specific primers and probe (Table 1), designed to amplify a 85 bp fragment of the NS1, were used in Real-Time PCR (qPCR) to quantify the viral load in all the samples yielding amplicons of expected size. Quantification was

performed using TaqMan Fast Advanced Master Mix (Invitrogen Ltd, Milan, Italy) in a 25-µl volume comprising 5 µl of extracted DNA and 20 µl of master mix. Primers and TaqMan probe were used at concentrations of 200 and 100 nM, respectively. Tenfold serial dilutions (from 10^0 to 10^8 copies) of a plasmid containing the synthetised 85-bp fragment were used in each PCR run.

Molecular screening for other feline enteric viruses

All faecal samples collected from cats with enteritis signs were tested molecularly for feline panleukopenia parvovirus (FPV), feline enteric coronavirus (FECV), feline kobuvirus (FeKoV) and norovirus (NoV) by quantitative or qualitative PCR and reverse transcription (RT)-PCR (Gut, Leutenegger, Huder, Pedersen, & Lutz, 1999; Buonavoglia et al., 2001; Vennema, de Bruin & Koopmans, 2002; Di Martino, Di Profio, Melegari, Marsilio & Martella, 2015).

Statistical Analysis

The association between clinical signs and presence of the virus in the enteric or respiratory samples was assessed using the Fisher's exact test using GraphPad Prism Software (https://www.graphpad.com/scientific-software/prism/). The significance level of the test was set at 0.05.

Genome sequencing and phylogenetic analysis

The amplicons were excised from the gel and purified using a QIAquick gel extraction kit (Qiagen GmbH, Hilden, Germany). The fragment was then subjected to direct sequencing using BigDye Terminator Cycle chemistry and 3730 DNA Analyzer (Applied Biosystems, Foster, CA). Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov) and FASTA (http://www.ebi.ac.uk/fasta33) with default values were used to find homologous hits.

Specific primers (Table 1) were designed by alignment of the full genomes of FeChPVs currently available in GenBank in order to generate additional genomic information on all the samples containing viral loads $>10^3$ DNA copies per 5 µl of template. Long PCRs were carried out using the La Taq DNA polymerase (Takara Bio Europe S.A.S. Saint-Germain-en-Laye, France). Amplicons were purified and cloned using a TOPO XL Cloning Kit (Life Technologies). Consensus sequences were generated by sequencing at least three clones for each PCR fragment. The alignment of the sequences was conducted using the MAFFT multiple alignment program version 7.388 plugin of the Geneious software (Biomatters Ltd., Auckland, New Zealand). Phylogenetic analyses were conducted in MEGA X software (Kumar, Stecher, Li, Knyaz & Tamura, 2018).

Sequence submission

The nearly complete genome (3,504 nt) of strains 313R/2019/ITA, 284R/2019/ITA and 49E/2019/ITA and the VP complete encoding gene (1,527 nt) of strains 261R/2019/ITA and 276R/2019/ITA were deposited in GenBank under accession numbers MW404251-3 and MW404254-5, respectively.

Results

Molecular investigations

Out of 89 enteric samples of collection A, FeChPV DNA was found in a total of 15 specimens with an overall prevalence of 16.9%. The detection rates were 36.8% (14/38) in diarrhoeic cats and 2.0% (1/51) in the control group (Table 2). The viral loads ranged from 1.0 x 10^2 to 1.5 x 10^4 DNA copies/5 µl of template (mean 3.4 x 10^3 DNA copies) in animals with AGE signs, whilst was 9.0 x 10^2 in the animals without AGE. The difference in FeChPV prevalence was strongly supported statistically ($p < 0.0001$, OR = 29,17, CI95% = [4,75; 314,5]) when comparing clinical cases and healthy animals enrolled as control. All the samples of collection A were tested molecularly for other enteric viruses. Whilst control animals tested negative for all the investigated pathogens, in diarrhoeic cats, 9/38 (23.7%) specimens tested positive to FPV, 2/38 (5.3%) to FECV, 2/38 (5.3%) to FeKoV and 2/38 (5.3%) to NoVs. All the FPV, FECV, FeKoV and NoV-positive samples were found coinfections with FeChPV (Table 3). In turn, FeChPV was detected alone in a diarrhoeic cat (7.1%, 1/14) and in a healthy animal. By screening the collection B (respiratory samples), FeChPV detection rate was 3.3% (6/183) in clinical cases (Table 2). The virus was found alone in 3/6 animals and in mixed infections with FHV-1 (2/6) or FCV (1/6). FeChPV was also found in 4.3% (6/140) animals of the control group, as single pathogen (4/6) or in dual (1/6) and triple (1/6) infections with either FCV or FHV-1 and *C. felis* (Table 4). Out of the 12 FeChPV positive cats, viral DNA was detected in ocular swabs from three cats (0.9%, 3/323) with (1/3) or without (2/3) URTD signs. Viral DNA was also detected in oropharyngeal swabs from seven animals (1.5%, 7/323) either with URTD signs (3/7) or healthy (4/7) and in paired ocular and oropharyngeal samples from two cats with URTD signs. The DNA viral load ranged from 1.5 x 10^1 to 3.8 x 10^5 with similar means (4.5 x 10^4 DNA copies) for cases and controls, with the highest mean titers of 2.4 x 10^4 DNA copies in oropharyngeal specimens, versus 3.5 x 10^2 DNA copies found in ocular swabs. Upon statistical

analysis, FeChPV prevalence did not significantly differ between cats with and without URTD signs.

Sequence analysis

The nearly full-length genome, encompassing the complete NS1 and VP encoding genes, was generated for a strain of enteric origin, 49E/2019/ITA (MW404253), and for two additional FeChPVs, 313R/2019/ITA and 284R/2019/ITA (MW404251 and MW404252), detected in oropharyngeal swabs of two cats with URTD signs, both which also tested positive in paired ocular swabs. By sequence comparison, the three strains displayed an overall nt identity of 98.8–99.4% to each other and of 98.7-99.3% to the two Canadian FeChPV prototypes (MN396757 and MN794869). Identity in the full-genome to the other members of the genus *Chaphamaparvovirus* ranged from 44.9% to 71.9%. The genome coding sequence of strains 49E/2019/ITA, 284R/2019/ITA and 313R/2019/ITA, excluding the terminal UTR regions was 3,442 nt in length, with 2 major open reading frames (ORFs) of 1,977 nt and 1,527 nt, coding respectively for the NS1 (658 aa) and for the VP capsid protein (508 aa). The termination of the NS1 encoding gene overlapped the start of the VP encoding gene by 62 nt. Similar to the genomic organization of other members of the genus *Chaphamaparvovirus* (Penzès et al., 2019), a minor ORF of 564 nt in length, coding for a predicted 187 aa nucleoprotein (NP), was located in position 1,352 → 1,915 nt overlapping the NS1 encoding gene. The NS1 protein of the three Italian strains contained the two conserved replication initiator (endonuclease) motifs ^{95}F**H**I**H**V/IMAL^{102} and ^{159}SLIA**Y**MC**K**^{156} (Smith and Kotin, 2000) and highly-conserved Walker motifs of the helicase domain, including Walker A (^{311}**G**CSNT**GK**S^{318}), B (^{349}IGVW**EE**^{354}), B′ (^{366}**K**QIFE**G**MECSIPV**K**^{379}) and C (^{391}IIM**T**T**N**^{396}) (Walker, Saraste, Runswick, & Gay, 1982; James et al., 2003). Upon sequence analysis of the VP protein, an 18 aa-elongation located at the N-terminus, encoded by a 54-nt region, was common to the three sequences generated in this study and to FeChPVs IDEXX-1 and 1/VRI protoypes previously

detected in Canada (Li et al., 2020). This feature has also been observed in other members of the genus *Chaphamaparvovirus*. It has been hypothesized that in addition to the canonical VP, some ChPVs possess a longer capsid protein, presumably expressed at low copies number (Penzes et al., 2019). As observed for canine ChPVs and other members of the subfamily *Hamaparvovirinae*, in the VP protein the conserved motifs of the phospholipase A2 (PLA2) were absent (Zádori et al., 2001).

Maximum likelihood-based phylogenetic analysis was performed using the NS1 protein of selected animal ChPVs belonging to the eight established *Chaphamaparvovirus* species (Pénzes et al., 2020) and of still unclassified strains. In the tree (Figure 1), the Italian ChPVs grouped tightly with the two FeChPV prototypes (97.7-99.2% aa identities) detected in Canada (Li et al., 2020) in a unique branch (bootstrap value 100%). FeChPV strains shared a common root with the unclassified ChPV strain Bat/CMR/14 (58.4-58.8% aa identities), identified in stools of a fruit bat in Cameroon (Yinda et al., 2018) and with CaChPVs (65.4-66.0% aa identities) classified into the novel species *Carnivore chaphamaparvovirus 1* (Fahsbender et al., 2019; Ji et al., 2020; Palombieri et aal., 2020). A nucleotide identity plot (Figure 2) of the complete genome of strain 313R/2019/ITA (MW404251) was compared with those of the feline strain IDEXX-1 (MN396757) (Li et al., 2020), the canine ChPV 1A-IDEXX1 (MH893826) (Fahsbender et al., 2019) and the bat strain Bat/CMR/14 (MG693107) (Yinda et al., 2018). The FeChPV 313R/2019/ITA showed high nucleotide conservation with the reference strain IDEXX-1 either throughout the NS1 (99.3% nt and 98.8% aa identities) and VP encoding genes (99.5% nt and 99.6% aa), whilst the feline viruses markedly differed from the ChPVs identified in dogs and bats (69.3-74.7% nt and 58.4-66.2% aa in the NS; 61.7-71.8% nt and 54.9-67.3% aa in the VP). In order to further investigate genetic features of FeChPVs, we analysed the VP protein of the Italian strains, including two additional complete capsid sequences (MW404254 and MW404255) obtained in this study from oropharyngeal specimens (261R/2019/ITA and 276R/2019/ITA) collected from two cats with URTD. In the VP based-tree (Figure 3), the five

FeChPVs detected in this study formed a unique cluster together with the prototypes identified in Canada (99.4-100% aa identities). Upon multiple alignment, genetic hallmarks associated with enteric or respiratory tropism or with geographical distribution of these novel feline parvoviruses could not be identified (data not shown).

Discussion

Feline panleukopenia parvovirus (FPV) infection has long been known in cats and the disease was already described in the early 20th century. Also, FPV for several decades has been the only recognized and studied feline parvovirus (Parrish, 1995). FPV is a member of the *Protoparvovirus* genus (subfamily *Parvovirinae*) classified within the species *Carnivore protoparvovirus 1* (Cotmore et al., 2014). FPV is currently regarded as the major causative agent of severe gastroenteritis in cats and it is included in feline core vaccination schedules globally (Di Martino et al., 2019). Advances in molecular diagnostics, including the development of broadly reactive consensus primers, genus- or family-specific and the introduction of NGS technologies, have enabled the identification and characterisation of several previously unknown parvoviruses in cats with and without disease, including novel members of the genus *Bocaparvovirus* (Lau et al., 2012; Ng et al., 2014; Zhang et al., 2014) and still unclassified bufavirus-like protoparvoviruses (Diakoudi et al., 2019). Although, many of these viruses have been occasionally identified in association with enteric, neurologic and respiratory signs (Garigliany et al., 2016; Yi et al., 2018; Diakoudi et al., 2019; Piewbang, Kasantikul, Pringproa, & Techangamsuwan, 2019), firm evidence in their possible pathogenic role in cats must be obtained.

In this investigation, a case–control study on cat samples of enteric and respiratory origin was performed in order to gather information on the possible role of the newly discovered FeChPVs in the development of enteric or respiratory disease. We observed a marked and significant higher prevalence (36.8%, 14/38) in the cohort of cats with diarrhoea with respect to a group of animals used as baseline (2.0%; 1/51). Also, molecular screening for other selected viral enteric pathogens, i.e., FPV, FECV, FeKoV and NoVs, revealed that FeChPV was the pathogen most frequently detected in clinical cases. This finding confirms previous observations on the possible aetiologic role of FeChPVs as enteric pathogen of cats (Li et al., 2020).

The ability of FPV to induce severe gastroenteritis has been repeatedly demonstrated either experimentally or observationally (Barrs, 2019). In our study, FPV was detected in 23.7% (9/38) of the animals with AGE, mostly in coinfections with FeChPV. Similarly, the majority of the FeChPV positive cats were also infected with other feline pathogens, whilst single infection was detected only in one animal with enteritis and in a control cat. Whether FeChPV may act as enteric pathogen alone or in synergism with other enteric viruses should be further investigated in larger and more structured investigations or in experimental infections.

When we analysed the results of collection B (respiratory samples), we detected the virus at low prevalence in both the cohorts (cases and controls), with the highest detection rate in healthy animals (4.3%, 6/140), and no significant association was found between FeChPV and URTD. FeChPV was detected alone or in coinfection with FCV, FHV-1 and *C. felis* either in cats with or without URTD.

Interestingly, although the prevalence of FeChPV in respiratory samples was lower than in enteric, upon quantification of the viral DNA, virus shedding was higher in oropharyngeal swabs than in enteric samples. Indeed, many of the viral sequences generated in our study were obtained from pharyngeal samples. Whether this virus possesses as preferential replication site the oropharyngeal tissue cannot be excluded and warrants further studies.

Upon sequence analysis of the nearly complete genome, the FeChPV strains 313R/2019/ITA, 284R/2019/ITA and 49E/2019 displayed > 98.5% nt identity to each other and to the two Canadian prototype viruses (Li et al., 2020). In the NS1-based tree the five feline strains formed a monophyletic cluster (97.7-99.2% aa identities) well distinct from the CAChPVs (58.4-66.2% aa identities) currently classified in the species *Carnivore chaphamaparvovirus 1*. Following strictly the ICTV classification criteria for species demarcation, the feline viruses detected in this study should be classified, together with strains IDEXX-1 and 1/VRI (Li et al., 2020), as a candidate novel species *Carnivore chaphamaparvovirus 2*

(https://talk.ictvonline.org/files/proposals/animal_dna_viruses_and_retroviruses/m/animal_dna_ec_approved/10408/download). Analysis of the VP capsid protein performed on five Italian strains and the two prototypes, revealed a strong sequence conservation among all the members of this novel species in the predicted VRs and confirmed the marked genetic divergence from CaChPVs of canine and feline origin of the species *Carnivore chaphamaparvovirus 1*. Whether this genetic diversity may reflect biological differences among the two group of viruses should be further investigated.

In conclusion, in this study evidence was collected on the circulation of novel ChPV in cat populations, confirming, after the initial identification in Canada, that FeChVP is a common component of feline virome. Our pilot epidemiological investigation also suggests that FeChPV infection is common in cats with AGE. Large-scale virological investigations and the inclusion of FeChPV in the diagnostic flowchart of feline enteric pathogens will be pivotal to fully address this issue and eventually conceive, if necessary, strategies to protect feline health.

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Ethical statement

Ethical statement is not applicable since sample collections were obtained from animals that were submitted to diagnostic investigations upon request of the owners.

Data availability statement

The data that support the findings of this study are openly available in the GenBank database at https://www.ncbi.nlm.nih.gov/nucleotide/ under accession numbers MW404251-5.

Conflict of interest statement

All Authors declare that there are no financial or other relationships that might lead to a conflict of interest. All authors have seen and approved the manuscript and have contributed significantly to the work.

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535 **Figure legends**

536 **Fig. 1 –** Phylogenetic analysis based on the aa sequence of the NS1 of FeChPV. The tree was
537 performed with a selection of ChPVs representative of each species of the genus
538 *Chaphamaparvavovirus* and still unclassified strains. Also, viruses representative of the genera
539 *Hepanhamaparvovirus, Penstylhamaparvovirus, Brevihamaparvovirus* and
540 *Ichthamaparvovirus* classified within the newly established subfamily *Hamapaparvovirinae*,
541 was included in the analyses. Phylogenetic analysis was constructed by using Maximum
542 Likelihood method based on the Poisson correction and supplying statistical support with
543 bootstrapping of 1000 replicates. Black triangles indicate the FeChPV strains detected in this
544 study. Evolutionary analysis was conducted in MEGA X (Kumar et al., 2018).

545

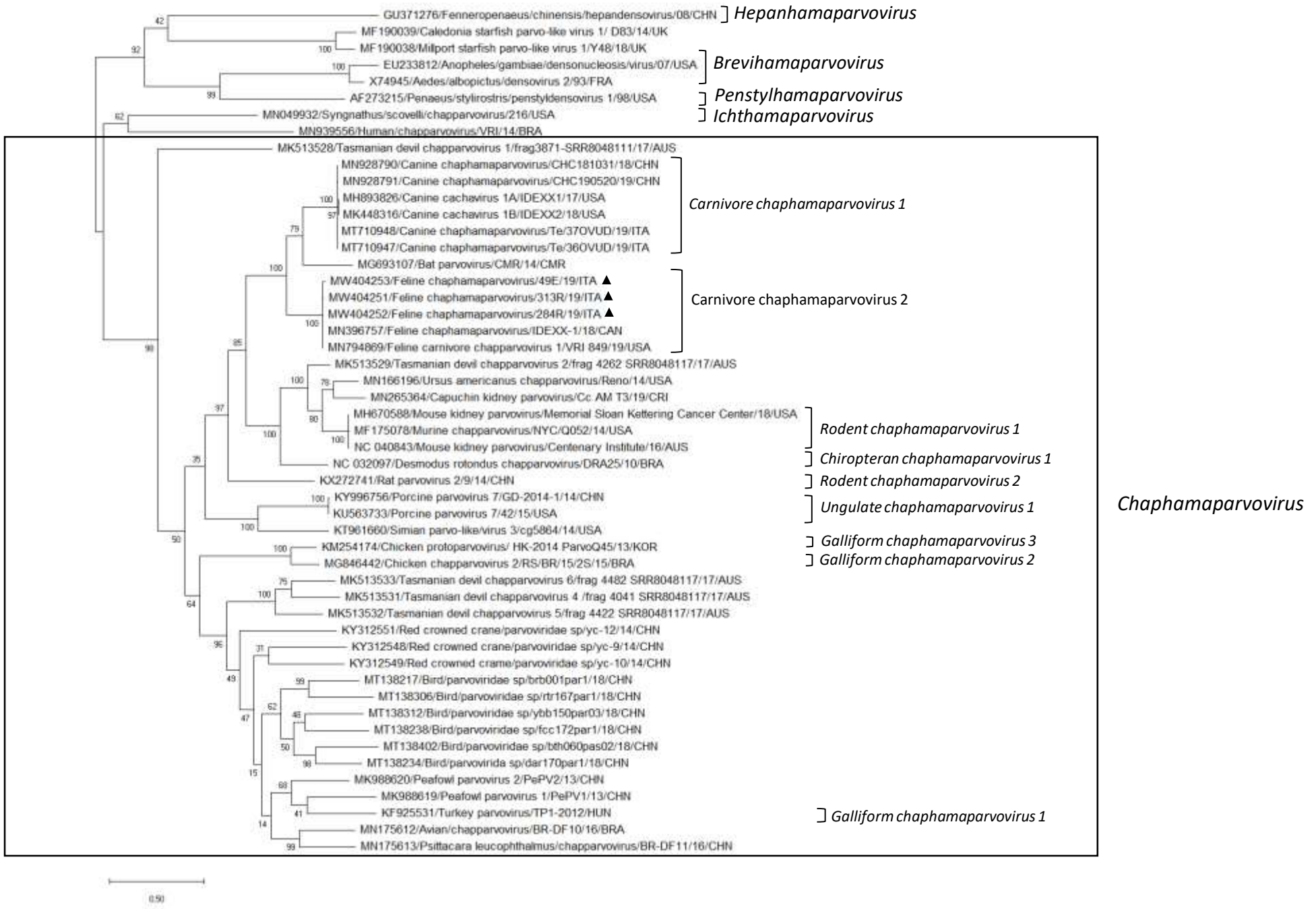
546 **Fig. 2** - Nucleotide identity plot comparing the nearly complete genome (3,442 nt) of strain
547 313R/2019/ITA (GenBank accession number MW404251) with those of the feline strain
548 IDEXX-1 (MN396757) (Li et al., 2020), the canine ChPV 1A-IDEXX1 (MH893826)
549 (Fahsbender et al., 2019) and the bat strain BtPV/CMR/14 (MG693107) (Yinda et al., 2018),
550 was performed by using Simplot (Lole et al., 1999) with a window size of 200 and a step size of
551 20 and with gap strip off and Hamming correction on.

552

553 **Fig. 3** - Phylogenetic analysis of the VP aa sequences of the FeChPV. Tree, based on the VP
554 capsid aa sequences of ChPVs detected to date in dogs and cats, was generated using Maximum
555 Likelihood method based on the Poisson correction. The bat strain Bat/CMR/14 (MG693107)
556 was used as outgroup. Black triangles indicate the FeChPV strains detected in this study.

557

Fig. 1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MF190038/Millport starfish parvo-like virus 1/Y48/18/UK
EU233812/Anopheles/gambiae/densonucleosis/virus/07/USA
X74945/Aedes/albopictus/densovirus 2/93/FRA
AF273215/Penaeus/stylirostris/penstyldensovirus 1/98/USA
MN049932/Syngnathus/scovelli/chapparvovirus/216/USA
MN939556/Human/chapparvovirus/VRI/14/BRA
MK513528/Tasmanian devil chapparvovirus 1/frag3871-SRR8048111/17/AUS
MN928790/Canine chaphamaparvovirus/CHC181031/18/CHN
MN928791/Canine chaphamaparvovirus/CHC190520/19/CHN
MH893826/Canine cachavirus 1A/IDEXX1/17/USA
MK448316/Canine cachavirus 1B/IDEXX2/18/USA
MT710948/Canine chaphamaparvovirus/Te/37OVUD/19/ITA
MT710947/Canine chaphamaparvovirus/Te/36OVUD/19/ITA
MG693107/Bat parvovirus/CMR/14/CMR
MW404253/Feline chaphamaparvovirus/49E/19/ITA ▲
MW404251/Feline chaphamaparvovirus/313R/19/ITA ▲
MW404252/Feline chaphamaparvovirus/284R/19/ITA ▲
MN396757/Feline chaphamaparvovirus/IDEXX-1/18/CAN
MN794869/Feline carnivore chapparvovirus 1/VRI 849/19/USA
MK513529/Tasmanian devil chapparvovirus 2/frag 4262 SRR8048117/17/AUS
MN166196/Ursus americanus chapparvovirus/Reno/14/USA
MN265364/Capuchin kidney parvovirus/Cc AM T3/19/CRI
MH670588/Mouse kidney parvovirus/Memorial Sloan Kettering Cancer Center/18/USA
MF175078/Murine chapparvovirus/NYC/Q052/14/USA
NC 040843/Mouse kidney parvovirus/Centenary Institute/16/AUS
NC 032097/Desmodus rotondus chapparvovirus/DRA25/10/BRA
KX272741/Rat parvovirus 2/9/14/CHN
KY996756/Porcine parvovirus 7/GD-2014-1/14/CHN
KU563733/Porcine parvovirus 7/42/15/USA
KT961660/Simian parvo-like virus 3/cg5884/14/USA
KM254174/Chicken protoparvovirus/ HK-2014 ParvoQ45/13/KOR
MG846442/Chicken chapparvovirus 2/RS/BR/15/2S/15/BRA
MK513533/Tasmanian devil chapparvovirus 6/frag 4482 SRR8048117/17/AUS
MK513531/Tasmanian devil chapparvovirus 4 /frag 4041 SRR8048117/17/AUS
MK513532/Tasmanian devil chapparvovirus 5/frag 4422 SRR8048117/17/AUS
KY312551/Red crowned crane/parvoviridae sp/yc-12/14/CHN
KY312548/Red crowned crane/parvoviridae sp/yc-9/14/CHN
KY312549/Red crowned crane/parvoviridae sp/yc-10/14/CHN
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MT138306/Bird/parvoviridae sp/rtr167par1/18/CHN
MT138312/Bird/parvoviridae sp/ybb150par03/18/CHN
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MT138402/Bird/parvoviridae sp/bth060pas02/18/CHN
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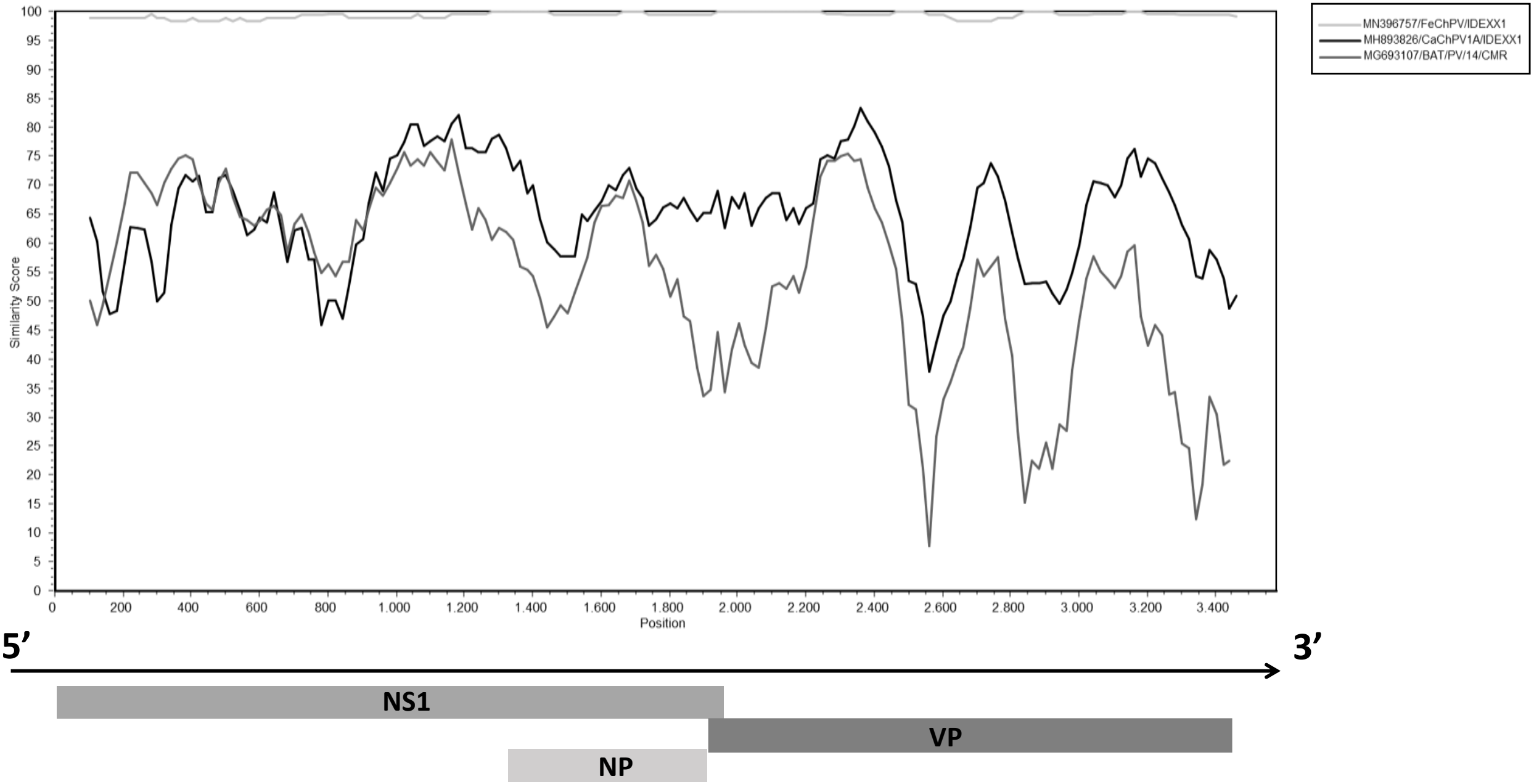
Hepanhamaparvovirus
Brevihamaparvovirus
Penstylhamaparvovirus
Ichthamaparvovirus
Carnivore chaphamaparvovirus 1
Carnivore chaphamaparvovirus 2
Rodent chaphamaparvovirus 1
Chiropteran chaphamaparvovirus 1
Rodent chaphamaparvovirus 2
Ungulate chaphamaparvovirus 1
Galliform chaphamaparvovirus 3
Galliform chaphamaparvovirus 2
Galliform chaphamaparvovirus 1
Chaphamaparvovirus

Fig. 2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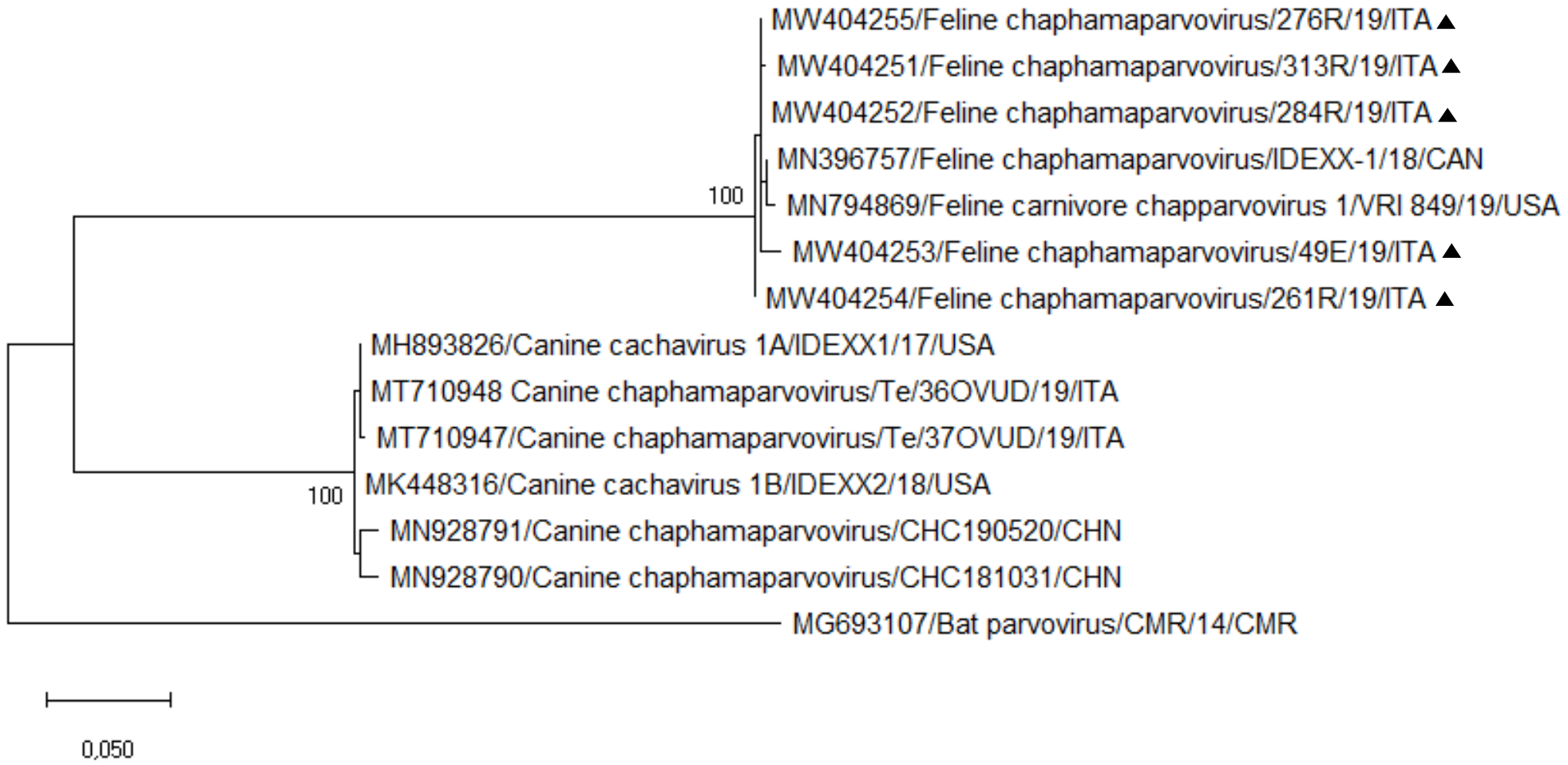
MG693107/BAT/PV/14/CMR
Similarity Score
Position
5'
3'
NS1
VP
NP

Fig. 3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MW404252/Feline chaphamaparvovirus/284R/19/ITA▲
MN396757/Feline chaphamaparvovirus/IDEXX-1/18/CAN
100
MN794869/Feline carnivore chapparvovirus 1/VRI 849/19/USA
MW404253/Feline chaphamaparvovirus/49E/19/ITA▲
MW404254/Feline chaphamaparvovirus/261R/19/ITA▲
MH893826/Canine cachavirus 1A/IDEXX1/17/USA
MT710948 Canine chaphamaparvovirus/Te/36OVUD/19/ITA
MT710947/Canine chaphamaparvovirus/Te/37OVUD/19/ITA
100
MK448316/Canine cachavirus 1B/IDEXX2/18/USA
MN928791/Canine chaphamaparvovirus/CHC190520/CHN
MN928790/Canine chaphamaparvovirus/CHC181031/CHN
MG693107/Bat parvovirus/CMR/14/CMR
0,050

Table 1 - List of primers used in this study. Nucleotide position refers to the sequence of the FeChPV isolate IDEXX-1 (GenBank accession no. MN396757).

Oligonucleotide	Position	Sequence (5' to 3')	Sense	Reference	Use
FeChPV_F1	2185-2204	GGTGCGACGACGGAAGATAT	+	Li et al., 2020	Diagnostic PCR
FeChPV_R1	2497-2516	CAACACCACCATCTCCTGCT	-	Li et al., 2020	Diagnostic PCR
FeChPV_F2	2206-2225	GCTGCAGTTCAGGTAGCTCA	+	Li et al., 2020	Diagnostic PCR/qPCR
FeChPV_R	2271-2290	ACCACAGCTAGCGACAGGAT	-	This study	qPCR
FeChPV_Pb	2235-2254	FAM-TGCTCCGACAGCTCAGGATC-BHQ1	+	This study	qPCR
FeChPV_140F	140-162	GTGGGAGCAAATACAGACAATGG	+	This study	Sequencing
FeChPV_678 F	678-697	ATGGAACGTAGCAGACGTGC	+	This study	Sequencing
FeChPV_1225R	1225-1246	TCTTGTTGTTTGATTCTAAATC	-	This study	Sequencing
FeChPV_1444F	1444-1464	CACATACTTGGTCACTAACCA	+	This study	Sequencing
FeChPV_1744R	1744-1765	TCGGCTAATTCAGGGCTTATTA	-	This study	Sequencing
FeChPV_2656F	2656-2677	TCTATATCATATTCAAATACTT	+	This study	Sequencing
FeChPV_2708R	2708-2729	TTATGCGTATTATTAGGATATA	-	This study	Sequencing
FeChPV_3269F	3269-3290	ACCCATCAAGTATAATGGAATT	+	This study	Sequencing
FeChPV_3338R	3338-3358	TGAACCATTTATTGTCATCTG	-	This study	Sequencing
FeChPV_4161R	4161-4182	AATTGAAGTATATCAGGTCATA	-	This study	Sequencing

Table 2 – Prevalence of FeChPV in enteric and respiratory samples

Collection A			
Clinical history	N. of sample	Positive samples (%)	Negative samples (%)
Healthy	51	1 (1.9%)	50 (98.1%)
Diarrhoeic	38	14 (36.8%)	24 (63.2%)
Total	89	15 (16.9%)	74 (83.1%)
Collection B			
Healthy	140	6 (4.3%)	134 (95.7%)
URTD	183	6 (3.3%)	177 (96.7%)
Total	323	12 (3.7%)	309 (96.3%)
Collection A + B			
Total	412	27 (6.6%)	385 (93.4%)

Table 3 – Detection of FeChPV in single and mixed infections with other feline enteric viruses

Collection A		Single infections	Mixed infections				
Clinical history	N. of samples	FeChPV	FeChPV + FPV	FeChPV + FeKoV	FeChPV + NoV	FeChPV +FPV + FECV	FeChPV+ NoV + FECV
Healthy	51	1	0	0	0	0	0
Diarrhoeic	38	1	8	2	1	1	1
Total	89	2	8	2	1	1	1

Table 4 - Detection of FeChPV in single and mixed infections with other feline pathogens causing respiratory signs

Collection B		Single infections	Mixed infections		
Clinical history	N. of samples	FeChPV	FeChPV + FCV	FeChPV + FHV-1	FeChPV +FHV-1 + *C. felis*
Healthy	140	4	1	0	1
URTD	183	3	1	2	0
Total	323	7	2	2	1