Full genome sequencing of dozens of new DNA viruses found in Spanish bat faeces

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ABSTRACT

Bats are natural hosts of multiple viruses, many of which have clear zoonotic potential. The search for emerging viruses has been aided by the implementation of metagenomic tools, which have also enabled the detection of unprecedented viral diversity. Currently, this search is mainly focused on RNA viruses, which are largely over-represented in databases. To compensate for this research bias, we analyzed fecal samples from 189 Spanish bats belonging to 22 different species using viral metagenomics. This allowed us to identify 50 complete or near-complete viral genomes belonging to the families *Adenoviridae*, *Circoviridae*, *Genomoviridae*, *Papillomaviridae*, *Parvoviridae, Polyomaviridae* and *Smacoviridae*. Of these, 28 could constitute new species, doubling the number of viruses currently described in Europe. These findings open the door to a more thorough analysis of bat DNA viruses and their zoonotic potential.

IMPORTANCE

Metagenomics has become a fundamental tool to characterize the global virosphere, allowing us to understand the existing viral diversity and its ecological implications, but also to identify new and emerging viruses. RNA viruses have a higher zoonotic potential, but this risk is also present for some DNA virus families. In our study, we have analyzed the DNA fraction of faecal samples from 22 Spanish bat species, identifying 50 complete or near-complete genomes of different viral families with zoonotic potential. This doubles the number of genomes currently described in Europe. Metagenomic data often produce partial genomes that can be difficult to analyse. Our work, however, has characterised a large number of complete genomes, thus facilitating their taxonomic classification and enabling different analyses to be carried out to evaluate their zoonotic potential. For example, recombination studies are relevant, since this phenomenon could play a major role in cross-species transmission.

Keywords: Bat viruses, DNA viruses, Metagenomics, Viral emergence, Viromics, Zoonotic viruses.

INTRODUCTION

Bats are the largest mammalian order after rodents, with around 1400 species distributed worldwide (1,2), and play an important role as pollinators, pest controllers, seed dispersers, and reforesters (3). However, they are also a natural reservoir for a wide variety of viruses. Indeed, some bat RNA viruses are at the origin of zoonotic diseases (4,5). Moreover, viruses may directly threaten bat populations, which can have important implications for ecosystem management (6,7). Bat-specific features may explain their propensity to carry viruses. For example, it has been suggested that evolution of metabolic mechanisms involved in flight capacity triggered pleiotropic effects related to pathogen immunity, thus increasing the susceptibility of bats to be asymptomatic carriers of viruses (8). Also, bats can form extremely large and densely populated colonies that tend to favour high rates of viral transmission (9). In this context, bat shelter disturbances may also increase contacts with humans or domestic animals, leading to an increased zoonotic risk. This threat has prompted the implementation of bat monitoring programs in several countries (10).

Numerous animal viruses have been discovered using metagenomics. These studies have significantly increased our knowledge of the global virosphere (11), and have enabled the identification of new and emerging viruses in various clinical and environmental samples (11,12). As of September 2023, the bat-associated virus database (i.e. DBatVir) included over 19,000 sequences, half of which originated from Asia, followed by Africa, with European origin samples representing less than 10%. In addition to this bias, most of the described bat viruses are RNA viruses, mainly coronaviruses, which account for more than half of the known sequences, while only 10% are DNA viruses. This over-representation of RNA viruses in databases is a consequence of their increased zoonotic potential (13), which has intensified efforts in their discovery over DNA viruses. Finally, the vast majority of viral sequences deposited in DBatVir are partial, usually from the viral polymerase or capsid genes, with full genome sequences being the exception.

Spain hosts over thirty bat species and stands out as one of the European countries with the highest number of described bat viruses, mainly RNA viruses. Specifically, DBatVir reports 298 viral sequences from Spain belonging to families *Rhabdoviridae*, *Adenoviridae*, *Coronaviridae*, *Herpesviridae*, *Papillomaviridae*, *Filoviridae* and *Picornaviridae.* These families include potentially zoonotic viruses such as lyssaviruses (14) and other rhabdoviruses (15), coronaviruses (16), herpesviruses (17), and a distant relative of ebolaviruses (18). In contrast, only four and 28 complete genomes of bat DNA viruses have been reported in Spain and Europe, respectively. To help correct this bias, we have used metagenomics to characterize the DNA virus fraction present in fecal samples from 189 bats, belonging to 22 species captured in different regions of Spain. Overall, the assembly of the viral reads obtained has enabled the recovery of 50 complete or nearly complete viral genomes belonging to the families *Adenoviridae*, *Circoviridae*, *Genomoviridae*, *Papillomaviridae*, *Parvoviridae, Polyomaviridae* and *Smacoviridae*, 28 of which represent novel DNA virus species.

MATERIALS AND METHODS

Study area and sample collection

Nylon mist nets and a harp trap (Austbat) were used to capture bats from different habitats that were abundant. Each captured animal was identified to species level, sexed, measured, weighed and briefly placed in cotton bags to recover fresh fecal samples. Fecal samples were obtained from 189 bats captured in seven Spanish regions (Cantabria, Castellón, Lugo, Murcia, Salamanca, Teruel, and Valencia; **Figure 1**) from May to October 2022. Of the 22 bat species 18, 3, and one belonged to the *Vespertilionidae*, *Rhinolophidae, and Molossidae* families, respectively. Samples from each individual were pooled in tubes containing 500 µL of 1X phosphate-buffered saline (PBS), kept cold initially, and then at -20 °C until they were transported to the laboratory and stored at -80 °C for further processing.

Sample processing and DNA extraction

A fraction of the samples from each of the 189 individuals was combined into a total of 25 pools, each containing between one and 15 samples from the same bat species (**Supplementary Table S1**). Fecal samples from each pool were homogenized in a Precellys Evolution tissue homogenizer (Bertin) in 2 mL tubes with 1.4 mm ceramic beads, adding 1 volume of 1X PBS to obtain a final volume of 1.5 mL. Homogenization consisted of 3 cycles of 30 s at 6500 rpm, with a 10 sec pause between cycles. Homogenates were centrifuged in two rounds at 20,000 g for 3 min at 4 °C. Supernatants were transferred to new tubes and filtered using Minisart cellulose acetate syringe filters with a 1.2 µm pore size (Sartorius). The filtrate was transferred to ultra-clean 2 mL tubes and 280 µL were collected for nucleic acid extraction using the QIAamp Viral RNA mini kit (Qiagen). The extract was eluted in a final volume of 40 µL and stored at -80 °C.

Sequencing and viral sequence detection

Extracted nucleic acids were used for library preparation using the Nextera XT DNA library preparation kit with 15 amplification cycles (Illumina) and subjected to paired-end sequencing in a NextSeq 550 device with the read length of 150 bp at each end. Reads were deduplicated, quality filtered with a quality trimming threshold of 20, and those reads below 70 nucleotides in length were removed using fastp v0.23.2 (19). De novo sequence assembly was performed using SPAdes v3.15.4 (20) with the meta option, and MEGAHIT v1.2.9 (21) using default parameters. The contigs assembled with either method were clustered to remove replicates or small replicates of larger contigs using CD-HIT v4.8.1 (22). Contigs shorter than 1000 nucleotides were removed. The resulting clustered sequences were then taxonomically classified using Kaiju v1.9.0 (23) with the subset of NCBI nr protein database containing archaea, bacteria and viruses, downloaded on June 6, 2023. All clustered sequences were also analyzed using Virsorter2 v2.2.4 (24) to detect viral contigs. In addition, viral contigs identified with Virsorter2 were analyzed with CheckV v1.0.1 (25) using the CheckV database v1.5 to further assess their quality. Finally, contigs corresponding to phages and those that could not be classified into a known viral family were discarded. The remaining contigs were selected based on their size, completeness, and the ability of the assigned virus family to infect vertebrates. In addition, all contigs related to the *Smacoviridae* and *Genomoviridae* families were also selected, as their ability to infect vertebrate cells has not been fully ruled out (26,27).

General phylogenetic analysis

Sequences similar to each contig of interest were searched using DIAMOND v2.0.15.153 (28) with the blastp option and the NCBI nr database downloaded on June 7, 2023. For each contig, the 100 closest sequences obtained from DIAMOND were retrieved and checked for association with vertebrate-infecting viruses, while protein domains were annotated using Interproscan v5.63-95.0 (29) with the Pfam database v35.0. Open reading frames (ORFs) were predicted using ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder). For those sequences assigned to viruses with the potential to infect vertebrates, a multiple sequence alignment was obtained using Clustal Omega v1.2.3 (30) or MAFFT v7.490 (31), depending on whether the alignment was amino acid or nucleotide based, respectively. Phylogenetic analyses were performed using IQ-TREE v2.0.3 (32), and model selection was done using the built-in ModelFinder feature (33). Branch support was estimated with 1000 ultra-fast bootstrapping replicates (34) and 1000 bootstrap replicates for the SH-like approximate likelihood ratio test. Coverage statistics for the viral contigs were calculated by remapping the trimmed and filtered reads to their associated contigs using Bowtie2 v2.2.5 (35). Where indicated, pairwise sequence identities were calculated with the Sequence Demarcation Toolkit (SDT) v1.2 (36), using MAFFT for sequence alignment. In addition, viral contigs were compared to NCBI databases using BLAST (37) to obtain identity values and refine annotations.

Family-specific phylogenetic analyses

For papillomaviruses, the Papillomavirus Episteme website (https://pave.niaid.nih.gov) (38) was initially consulted using the L1 Taxonomy Tool Analysis, which performs a pairwise alignment with the papillomavirus sequences available in this database. Then, the E1, E2, L2 and L1 nucleotide sequences from 206 representative papillomaviruses, assigned to the TaxId 151340, were downloaded from NCBI, concatenated, and aligned with MAFFT, using the GTR+F+I+G4 model to construct a maximum likelihood (ML) tree. In addition, to carry out the coevolution analysis, the associated host phylogeny was downloaded from TimeTree (www.timetree.org) (39).

For the phylogenetic analysis of viral contigs identified as polyomaviruses, large tumor antigen (LTAg) amino acid sequences were aligned with Clustal Omega and ambiguous regions in the alignment were trimmed with trimAl v1.2rev59 (40) using the *gappyout* parameter. For the *Parvoviridae* family, the analysis was done using the complete NS1 amino acid sequence and 126 members of the *Parvovirinae* subfamily (41). Sequences were aligned using Clustal Omega and the ML tree was computed using LG+F+I+G4 as the amino acid substitution model with 1000 ultrafast bootstrap replicates. For the family *Adenoviridae*, the analysis was performed using the Hexon and DNA-dependent DNA polymerase sequences. Sequences were aligned using Clustal Omega and the ML tree was computed using LG+F+I+G4 as the amino acid substitution model with 1000 ultrafast bootstrap replicates. For the Cressdnaviricota phylum (i.e. *Circoviridae*, *Smacoviridae*, and *Genomoviridae* families), the amino acid sequence of the replication-associated protein (i.e. Rep) was used following previous work (27,42,43). For *Genomoviridae* and *Smacoviridae* families, Rep alignments were also trimmed using the *gappyout* option from trimAl. In addition, to perform genome-wide pairwise analyses, all genomic sequences were first reoriented, optimizing the position of the putative origin of replication (*ori*) using MARS (44).

Amplification of viral sequences by PCR

Those viruses identified as having a high probability of infecting bats, and not originating from other sources such as diet, were analyzed by PCR. Thus, viruses assigned to the *Smacoviridae* and *Genomoviridae* families were excluded from this analysis (26,27). For this purpose, specific primers were designed to amplify a small region of about 500 bp for each virus of interest (**Supplementary Table S2**). Initially, nucleic acids were extracted individually from each animal sample for the pools of interest using the QIAamp Viral RNA mini kit (Qiagen), and DNA was eluted in 30 µL. Then, 1 µL was analyzed by PCR using NZYTaq II Green Master Mix (NZYTech) and specific primers for each virus of interest on all individual samples from the pool where it was detected. To assign which samples were positive for each target virus and their geographical location, amplification products were visualized by electrophoresis using a 1% agarose gel with Green Safe Premium (NZYTech).

RESULTS AND DISCUSSION

We obtained feces from 189 bats belonging to 22 species. These samples were processed in 25 pools, each pool containing exclusively samples belonging to the same species. Illumina sequencing from DNA samples generated between 4.9 and 23 million raw reads per pool (**Supplementary Table S3**). Quality-filtered reads were de novo assembled, and the resulting contigs were analyzed to identify viral sequences. As a result, 35,607 viral contigs over 1kb were obtained, of which 1053 were complete or nearly complete. Of these metagenome-assembled viral genomes (MAVGs), we focused on 50 belonging to five different families of vertebrate viruses (*Polyomaviridae*, *Papillomaviridae*, *Adenoviridae*, *Parvoviridae, and Circoviridae*), as well as two little studied families (*Smacoviridae* and *Genomoviridae*). These 50 MAVGs were identified in individuals from 11 different bat species (**Figure 2**). Eight of the MAVGs showed >85% sequence identity with previously described viruses at >90% coverage, while 42 corresponded to potential new viruses (**Supplementary Table S4**). The proposed names and accession numbers for these MAVGs are shown in **Supplementary Table S4**. In each of the sections below, we discuss whether these MAVGs can be considered new viral species according to the criteria established for each viral family.

Novel members of the family *Papillomaviridae*

Four MAVGs showed a genomic organization typical of papillomaviruses: MAVG3, MAVG45, MAVG46, and MAVG49. These were detected in fecal samples from *Barbastella barbastellus* (pool P8), *Pipistrellus kuhlii* (P20), *Rhinolophus ferrumequinum* (P24), and *Plecotus austriacus* (P26), respectively (**Figure 2**; **Supplementary Table S4**). All encoded four early genes (E6, E7, E2, and E1) and two late genes (L2, L1) located on the same coding strand (6), with non-coding regions between L1 and E6 genes, and between early and late genes. In addition, MAVG49 contained a small intergenic region between the L1 and L2 genes.

To ascertain the precise geographic origin of each virus, we used sequence-specific primers to test by PCR each individual sample from the pools containing these four MAVGs (**Supplementary Tables S1, S2 and S4**). This showed that MAVG3 (identified in pool P8) was present in three animals captured in Northern Spain (Begonte, Lugo), whereas MAVG49 (pool P26) was detected in a single individual captured in a nearby

location (Outeiro de Rei, Lugo), and MAVG 45 (pool P20) was present in three animals captured in Eastern Spain (Fontanars del Aforins, Valencia). Finally, MAVG46 was found in pool P24, which only included two animals from Fuente Álamo (Murcia), so no further analysis was required in this case. Despite the small number of animals sampled, these results suggest that at least some of the papillomaviruses identified are widely distributed in the populations tested.

Papillomavirus taxonomy is based on nucleotide sequence identity across the L1 gene (45). Two papillomaviruses belong to the same genus if they share more than 60% sequence identity, whereas sequences that share >70% identity are considered viral variants of the same species. MAVG3 and MAVG45 shared 71.3% and 77.4% sequence identity, respectively, with Eptesicus regulus papillomavirus (Acc. MT766314.1), an unclassified papillomavirus from Australian bats, and thus were variants of the same species. We note that both MAVGs were detected in different bat species, also distinct from the Australian variant, which reveals cross-species transmission. In addition, this is the first time that variants of the genus including this species have been described outside Australia. MAVG49 shared 73.6% sequence identity with Eptesicus serotinus papillomavirus 1 and 3 (Acc. NC_038518.1 and KC858265.1, respectively), both isolated from Spanish bats, and was therefore considered a new type of the same species. In this case, although the geographical location is common, these viral variants have been detected in different bat species, again showing cross-species transmission. This demonstrates that at least bat papillomaviruses have the ability to infect phylogenetically closely related hosts. Finally, MAVG46 was considered a new papillomavirus species, since it showed 68.9% sequence identity with the closest sequence, Rhinolophus ferrumequinum papillomavirus 1 (Acc. NC_038527), identified in the same bat species.

A recent study has shown direct evidence of virus-host coevolution in a subclade including several bat and other mammalian papillomaviruses (46). Since our MAVGs were embedded in this subclade (global tree not shown), we decided to replicate this previous analysis to test whether the observed cross-species transmission events could compromise coevolution detection. To do so, the subclade of interest was selected from the global papillomavirus tree, as previously described (46), and a tanglegram including the obtained tree with the cytochrome B nucleotide sequences of the associated host species was then used (**Figure 3**). The Wasserstein distance (46,47) between the host and virus phylogenetic trees was 0.25 (two trees are topologically identical when the Wasserstein distance is 0). In addition, we used the Procrustean Approach to Cophylogeny (PACo) (48) to assess the congruence between the viral and host phylogenies. The observed best-fit Procrustean superposition (3.78) lied outside the 95% confidence interval of the ensemble of 1000 network randomizations in the null model. These results confirm that, at the local level, co-speciation may be a determining factor in the evolution of papillomaviruses, as previously shown (46). Globally, however, the evolutionary history of papillomaviruses is more complex, with multiple polyphyletic lineages infecting the same host, such as primates, rodents or dolphins (6). For example, the clade grouping several genera of bat papillomaviruses also included a human papillomavirus (human papillomavirus type 41; **Figure 3**). This suggests that other evolutionary mechanisms, like intra-host divergence or niche adaptation, likely contribute to the papillomavirus phylogenetic tree (49,50).

Novel members of the family *Polyomaviridae*

Three polyomavirus genomes, MAVG25, MAVG34, and MAVG50, were detected in fecal samples from *Myotis daubentonii* (pool P11), *Eptesicus serotinus* (pool P14), and *Plecotus auritus* (pool P27), respectively (**Figure 2**; **Supplementary Table S4**). MAVG34 and MAVG50 showed a genome organization typical of polyomaviruses, presenting early expressed regulatory genes (encoding LTAg and small tumor antigen (STAg)) and late expressed protein genes (VP1 and VP2) (51). MAVG25, however, has a slightly different organization, as LTAg and STAg protein domains are in the same ORF, whereas they are usually found in different ORFs. As above, PCR was carried out for individual samples to reveal precise geographical location of these viruses (**Supplementary Tables S1, S2, and S4**). This showed that all were present in very close locations in Northwestern Spain. Specifically, MAVG34 and MAVG50 were detected in two individual samples obtained from Outeiro de Rei (Lugo) and pertaining to pools P14 and P27, respectively, while MAVG25 was detected in two individual samples from pool P11 obtained at another location from the same province (Rábade, Lugo; **Supplementary Table S1**).

According to the phylogenetic analysis of the LTAg sequence (51,52), these three MAVGs belong to the genus *Alphapolyomavirus*, and more specifically, they are located within a monophyletic group characterized by the absence of the VP3 protein and a long VP1 (53) (**Figure 4**). This group is also known as Merkel cell polyomavirus group or VP3-less clade (53), and includes numerous viruses from bats, but also from many other mammals, such as various primates, including humans. Since the three MAVGs showed less than 85% sequence identity in LTAg with other polyomaviruses, they represent new species according to ICTV criteria. Specifically, MAVG25 showed a peak sequence identity of 73.5% with Myotis davidii polyomavirus (Acc. LC426673.1), an unclassified polyomavirus isolated from *Myotis davidii* in China. MAVG34 showed a maximum sequence identity of 78.4% with an unclassified polyomavirus isolated from *Pipistrellus pipistrellus* in China (Acc. LC426677.1). Finally, MAVG50 showed the highest sequence identity (76.10%) with an unclassified bat polyomavirus isolated from *Tadarida brasiliensis* in Brazil (Acc. NC_026015.1).

Infections of different species of horseshoe bats by the same polyomavirus have been described (54), providing evidence that short-range host-switching of polyomaviruses is possible in some cases. Thus, the reported new polyomaviruses are unlikely to be able to infect human cells, but their characterization may help elucidate the evolutionary history of polyomaviruses and clarify the conditions for important host-switching events.

Novel members of the family *Parvoviridae*

Two parvovirus genomes, MAVG43 and MAVG48 were detected in fecal samples from *Pipistrellus kuhlii* (pool P19) and *Rhinolophus ferrumequinum* (P24), respectively (**Figure 2**; **Supplementary Table S4**). Both MAVGs showed the typical parvovirus genome organization, encoding the nonstructural protein 1 (NS1), and a single capsid protein (VP) (55). PCR using NS1-specific primers (**Supplementary Tables S1, S2, and S4**) led to detection of MAVG43 in a single sample from pool P19 collected in Eastern Spain (Fontanar dels Aforins). MAVG48 was detected in a pool containing only two individuals captured in the same location (Fuente Álamo, Murcia) and hence no PCR was done to identify the virus in individual samples.

Parvovirus species are defined using an 85% identity threshold for the NS1 amino acid sequence (41). MAVG43 belongs to genus *Protoparvovirus* within the *Parvovirinae* subfamily (**Figure 5**), but BLASTp analysis of its NS1 sequence against protoparvoviruses only showed a peak sequence identity of 44.3% and a coverage of 86% with Protoparvovirus carnivoran1 (Acc. MT815972.1). Consequently, MAVG43 is a new protoparvovirus species. MAVG48 was assigned to genus *Dependoparvovirus* (**Figure 5**), also included in the *Parvovirinae* subfamily, and showed a maximum sequence identity of 98.4% with Adeno-associated virus Croatia cul1_12 (Acc. QHY93489.1) in the NS1 protein sequence. Therefore, MAVG48 is a very close variant of a virus described in another European country, but it should be noted that this is the first time that a dependoparvovirus is detected in Spanish bat populations.

Both *Protoparvovirus* and *Dependoparvovirus* genera contain viruses from different mammals, such as bats, rodents and primates. Within the genus *Dependoparvovirus*, there are adeno-associated viruses that infect humans but are considered as non-pathogenic (56). Furthermore, human-associated protoparvoviruses have been detected in recent years, mostly in metagenomic fecal studies. Some of these protoparvoviruses have been found in individuals with gastrointestinal disease (57). Parvoviruses have undergone species jumps and also exhibit high levels of genome variation, similar to RNA viruses (58). The new protoparvovirus described here, Pipistrellus kuhlii parvovirus, was associated with a bat species that lives in close proximity to humans and their pets. This close contact is a risk factor for zoonotic infections, given that protoparvovirus host-switching events are believed to involve cats, dogs, and raccoons (58,59).

Novel members of the family *Adenoviridae*

Two adenovirus genomes, MAVG44 and MAVG47, were detected in pooled fecal samples from *Pipistrellus kuhlii* (pool P20) and *Rhinolophus ferrumequinum* (P24), respectively (**Figure 2**; **Supplementary Table S4**). Both viruses belonged to the genus *Mastadenovirus* (**Figure 6**) and showed the typical genome organization of this group. MAVG44 had a GC content of 55%, in the range described for mastadenoviruses (60), and presented inverted terminal repeats (ITR) of 32 bp at both ends of the genome and 22 ORFs with putative coding sequences. As expected for the E3 region of non-primate mastadenoviruses, which is usually much simpler and shorter (61), MAVG44 showed a putative E3 region including a single ORF of 3671 nt. Although no protein domains were detected in this ORF, a BLASTp search showed 30% sequence identity and 90% coverage with the E3L protein of an Australian bat mastadenovirus (Acc. QGX41974.1). PCR analysis showed that MAVG44 was present in three individuals from pool P20 sampled at the same location (Fontanar dels Aforins; **Supplementary Tables S1, S2, and S4**). MAVG47 was found in a pool of two samples from the same location (Fuente Álamo, Murcia), so no PCR testing was done in this case. This genome had a GC content of 45.7%, and presented ITRs of 58 bp at both ends of the genome and 22 ORFs with putative coding sequences. MAVG47 showed a single ORF for E3, which contained immunoglobulin domains (IPR007110) and exhibited 30.7% amino acid sequence identity and 97% coverage with the E3L protein of bat mastadenovirus WIV9 (Acc. YP_009246364.1).

Taxonomic classification of mastadenoviruses is usually done using a non-structural protein, such as DNA polymerase, and a structural protein (e.g. hexon protein) (62). ML trees for two different proteins showed that MAVG44 and MAVG47 clustered with non-primate adenoviruses (**Figure 6**). Species definition is a complex task in mastadenoviruses, as it depends on several factors, such as phylogenetic distance, genome organization, or host range, among others. In any case, for MAVG44, a BLASTp search of the DNA polymerase and hexon amino acid sequences showed a maximum sequence identity of 76.6% and 83%, respectively, with a mastadenovirus found in *Chalinolobus gouldii*, an Australian bat (Acc. QGX41974.1). MAVG47 showed a peak sequence identity of 81.6% and 82.9% for the DNA polymerase and hexon sequences, respectively, with bat mastadenovirus WIV10 (Acc. YP_009246389.1), a member of the bat mastadenovirus C species isolated from *Rhinolophus sinicus* in China.

Adenoviruses are believed to be highly abundant in European bats (63), but additional sequencing efforts would be needed to achieve a more genome-wide characterization of these viruses. In this study, we have identified two complete genomes. However, due to the large genome size of adenoviruses, metagenomic studies typically yield partial sequences (63,64). Previous work based on partial hexon sequences has suggested that cross-species transmission may have occurred between human and bat hosts (65). Obtaining complete genomes may help to address this more thoroughly and to identify the origins of recombination events that could play a major role in cross-species transmission (66).

Novel members of the family *Circoviridae*

Eight circovirus MAVGs were detected in four pooled fecal samples from *Barbastella barbastellus* (P8; MAVG4 and MAVG5), *Myotis mistacinus* (P9; MAVG10, MAVG11, and MAVG12), *Myotis capaccinii* (P13; MAVG31 and MAVG32), and *Eptesicus serotinus* (P14; MAVG35; **Figure 2**; **Supplementary Table S4**). All MAVGs included two bidirectional major (>600 nt) ORFs encoding the Rep and capsid (Cp) proteins, and genomes sizes ranged between 1.73 and 2.17 kb, the expected size for a circovirus genome (67). In addition, the conserved nona-nucleotide motif marking the *ori* was detected in the intergenic region located between the 5' ends of both ORFs. PCR analysis showed that MAVG4, MAVG10, MAVG11, and MAVG12 were present in only one individual, in all cases at different locations in the province of Lugo (**Supplementary Tables S1, S2, and S4**). MAVG5 was detected in four individuals, three from Lugo and one from Salamanca. In addition, MAVG31 was present in five individuals, one from Lugo and four from Murcia. Finally, MAG32 and MAVG35 were detected in three individuals each, sampled from Murcia and Lugo, respectively.

The family *Circoviridae* includes two genera, and taxa assignment to each genus is based on the location of the *ori*, which is found on the Rep or CP coding strand for the genera *Circovirus* and *Cyclovirus*, respectively. Using this criterion together with Rep phylogenetic analysis, five MAVGs were assigned to the *Cyclovirus* genus and three to the *Circovirus* genus (**Figure 7**). Given that the species demarcation threshold is 80% genome-wide nucleotide sequence identity (67), the three MAVGs assigned to *Circovirus* genus could be considered new species. Concerning cycloviruses, MAVG10 and MAVG12 showed a genome-wide maximum sequence identity of 92.1 and 98.9% with cycloviruses isolated from chicken feces (Acc. MN379598.1 and NC_040639.1,

respectively), whrereas MAVG31 presented an 82.2% with a human associated cyclovirus (Acc. MZ201305.1).

The zoonotic potential of members of the family *Circoviridae* remains unknown. Most of the functional information available about this family comes from the study of a few members of the genus *Circovirus*, mainly porcine circoviruses (68) and beak and feather disease virus (69). In the case of the genus *Cyclovirus*, however, which has no cultured representatives, very little is known about its infectivity, transmission or host range. Hence, the identification by metagenomics of members of this family in bat feces does not allow us to ascertain whether these are true bat viruses, particularly for cycloviruses.

Novel members of the family *Smacoviridae*

Seven MAVGs belonging to this family were detected in two pooled fecal samples from *Myotis mistacinus* (P9; MAVG13, MAVG14, MAVG15, MAVG16, MAVG17, and MAVG18), and *Eptesicus serotinus* (P14; MAVG36; **Figure 2**; **Supplementary Table S4**). All MAVGs contained two ORFs encoding the Rep and capsid proteins in an ambisense orientation, and genomes sizes ranged between 2.4 and 2.9 kb, as expected for a smacovirus genome (70). In addition, all MAVGs also showed the *ori* nonanucleotide motif described in the *Smacoviridae* family and two intergenic regions (42), except MAVG15, which only presented one. The genus demarcation threshold for this family is 40% Rep amino acid sequence identity (42). Accordingly, all MAVGs belonged to the genus *Porprismacovirus*, except MAVG36, which was assigned to the genus *Inpeasmacovirus* (**Figure 8**). According to the 77% genome-wide pairwise sequence identity criterion used for delimitating species (70), all MAVGs corresponded to new species, although the high sequence identity shared by MAVG14 and MAVG16 (80.7%) grouped them as members of the same species.

The biology of smacoviruses is largely unknown, as they have not been cultured to date and have simply been associated with animals, insects, and even archaea (26,70). Most of the members of this family have been detected in metagenomic studies of animal fecal samples (70), with a few being detected in domestic animal serum and tracheal swab samples (71,72). Therefore, it has not been possible to assign a specific host and it is not known whether these viruses can be pathogenic for mammals or vertebrates.

Novel members of the family *Genomoviridae*

We detected 24 MAVGs belonging to *Genomoviridae* family in seven pooled fecal samples from seven bat species (**Figure 2**; **Supplementary Table S4**). Genome sizes ranged between 2.02 and 2.36 kb, as expected for genomoviruses (73). MAVGs showed one or two ORFs encoding the Rep and one ORF encoding the capsid protein, except for MAG9, which had two. In accordance with the genus demarcation criterion, which is based on Rep amino acid sequence phylogeny, 10, 9, and 5 MAVGs were assigned to the genera *Gemycircularvirus*, *Gemykolovirus*, and *Gemykrogvirus*, respectively (**Figure 9**). The species delimitation threshold is a 78% pairwise sequence identity genome-wide (27). Accordingly, 12 new species were identified, some of which included more than one MAVG (**Figure 9**; **Supplementary Table S5**). MAVG19, MAVG21, MAVG27, and

MAVG39, showed >99% genome sequence identity with other genomoviruses previously described (**Supplementary Table S4**).

The first known genomovirus was isolated from the plant pathogenic fungus *Sclerotinia sclerotiorum* (73). Since then, more than 400 complete genomes have been described in metagenomic studies, and 10 genera have been defined (27). Members of this family have been found in insects, plants, fungi, and vertebrates (including humans), and the true extent of their host range remains unknown, as does their involvement in a pathogenic role.

CONCLUSIONS

The starting point for the study of viral emergence is the characterisation of wildlife diversity. This has prioritized tropical regions, where land-use alterations, high wildlife diversity, and bush meat consumption are believed to increase disease emergence risk (74). It should be noted, though, that some emerging viral diseases have not originated in tropical areas (75,76). Wildlife biodiversity is lower in Europe, which implies a lower zoonotic potential, but also suffers from a serious problem of destruction and transformation of different habitats, particularly in Spain, which promotes closer contacts between humans and wild mammals. Indeed, animal-to-human viral transmission events regularly occur in Europe (77). It is therefore necessary to undertake studies to characterise wildlife diversity in this region, and to develop local viral surveillance programs, which will improve our ability to respond to potential outbreaks. For this purpose, bats, due to their high potential to harbour zoonotic viruses, are the primary action target.

RNA viruses have a higher zoonotic potential than DNA viruses (78). However, this risk is also present for some DNA virus families, where there are examples of viruses with zoonotic potential and which represent a threat to both animal populations and public health. Our study design was not intended to draw epidemiological conclusions, but primarily to reflect the existing diversity of DNA viruses in Spanish bats. However, it should be noted that, despite the small sample size, half of the viruses analyzed by PCR were present in more than one individual, suggesting that these infections were not exceptional but could be characterised by high population prevalence. In addition, our results also point to the need to study DNA viruses to better understand key aspects, such as transmission dynamics or host range. This will allow us to discern their true zoonotic potential and to establish surveillance strategies, as is currently being considered for RNA viruses.

Acknowledgments

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Data availability

The raw sequence reads were deposited in the Sequence Read Archive of GenBank under accession numbers SRR27912327-51. The MAVGs described in this study, which corresponded to complete or nearly complete genomes, were deposited in Genbank under accession numbers PP410048-97 (**Supplementary Table S4)**.

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Figure 1. Sampling points throughout Spain. The number of individuals captured in each area is indicated in parentheses.

Figure 2. Distribution of MAVGs per bat species/pool. Viral families are shown in different colours.

Figure 3. Optimized tanglegram between a papillomavirus subclade of the ML tree obtained from concatenated E1, E2, L2, and L1 nucleotide sequences (46), and associated host species. The host species tree was downloaded from www.timetree.org. The newly described viruses are highlighted in red boxes. Bootstrap values are shown at nodes. Both trees are rooted at midpoint.

Figure 4. ML tree of the family *Polyomaviridae* using 135 RefSeq LTAg amino acid sequences (NCBI TaxId: 151341). Taxonomic groups are collapsed by genus. Only taxa belonging to the genus *Alphapolyomavirus* are explicitly indicated, and the group known as VP3-less clade is highlighted in blue. Taxa are denoted by Genbank protein accession number and virus name, and novel viruses are labelled in red. Phylogenetic analysis was done using substitution model LG+F+I+G4. SH-aLRT and bootstrap values higher than 80 and 95, respectively, are indicated with red circles. The tree is rooted at midpoint. The scale bar indicates the evolutionary distance in amino acid substitutions per site.

Figure 5. ML tree of the Parvovirinae subfamily using 126 NS1 amino acid sequences. Taxonomic groups are collapsed by genus except for *Dependoparvovirus* and *Protoparvovirus* genera. Taxa are denoted by Genbank protein accession number and virus name, and novel viruses are labelled in red. Phylogenetic analysis was done using substitution model LG+F+I+G4. SH-aLRT and bootstrap values higher than 80 and 95, respectively, are indicated with red circles. The tree is rooted at midpoint. The scale bar indicates the evolutionary distance in amino acid substitutions per site.

Figure 6. ML trees of the *Adenoviridae* family using DNA polymerase (A) and hexon (B) amino acid sequences from 73 representative members. Taxonomic groups are collapsed by genus, except for the genus *Mastadenovirus*. Taxa are denoted by Genbank protein accession number and virus name, and novel viruses are labelled in red. Phylogenetic analyses were done using substitution model LG+F+I+G4. SH-aLRT and bootstrap values higher than 80 and 95, respectively, are indicated with red circles. The tree is rooted at midpoint. The scale bar indicates the evolutionary distance in amino acid substitutions per site.

Figure 7. ML tree of the *Circoviridae* family based on Rep amino acid sequence. Taxa are denoted by Genbank accession number and virus name, and viruses found in this study are indicated in red, while new species are indicated by an asterisk. Sequences were downloaded from the ICTV Circoviridae data resources, (27 November 2023). In addition, 3 RefSeq sequences (NC_076479, NC_040639.1 and BBI18985.1) were added to illustrate its similarity with novel MAVGs. Phylogenetic analysis was done using substitution model LG+F+R6. SH-aLRT and bootstrap values higher than 80 and 95, respectively, are indicated with red circles. The tree is rooted to define monophyletic

groups of each family genus. The scale bar indicates the evolutionary distance in amino acid substitutions per site.

Figure 8. ML tree of the family *Smacoviridae* based on 215 Rep amino acid sequences. Taxonomic groups are collapsed by genus, except for *Porprismacovirus* genus, and some non-illustrative clades within this genus. Taxa are denoted by Genbank accession number and virus name, and novel viruses are labelled in red. Phylogenetic analysis was done using substitution model LG+F+I+G4. SH-aLRT and bootstrap values higher than 80 and 95, respectively, are indicated with red circles. The tree is rooted at midpoint. The scale bar indicates the evolutionary distance in amino acid substitutions per site.

Figure 9. ML tree of the family *Genomoviridae* based on 94 representative amino acid sequences of Rep gene. Taxonomic groups are collapsed by genus, except for those genera where new viruses are identified. Taxa are denoted by Genbank accession number and virus name, and novel viruses are labelled in red, indicating with an asterisk those that are defined as new species. When a new species includes more than one novel MAVG (see **Supplementary Table S5**), only one is indicated with an asterisk. Phylogenetic analysis was done using substitution model LG+F+I+G4. SH-aLRT and bootstrap values higher than 80 and 95, respectively, are indicated with red circles. The tree is rooted at midpoint. The scale bar indicates the evolutionary distance in amino acid substitutions per site.

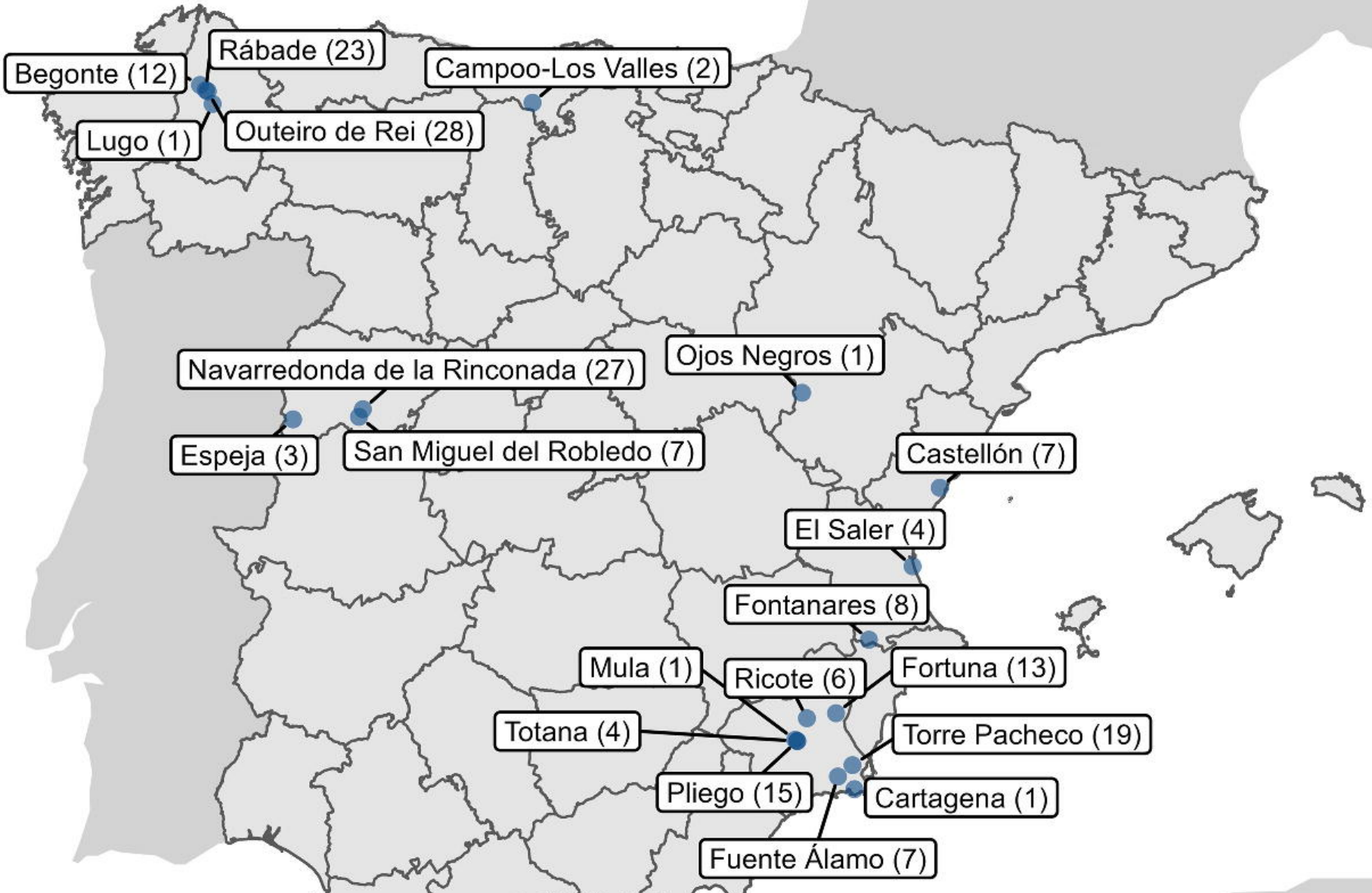
Supplementary Table S1. Bat species, collection sites and pool distributions.

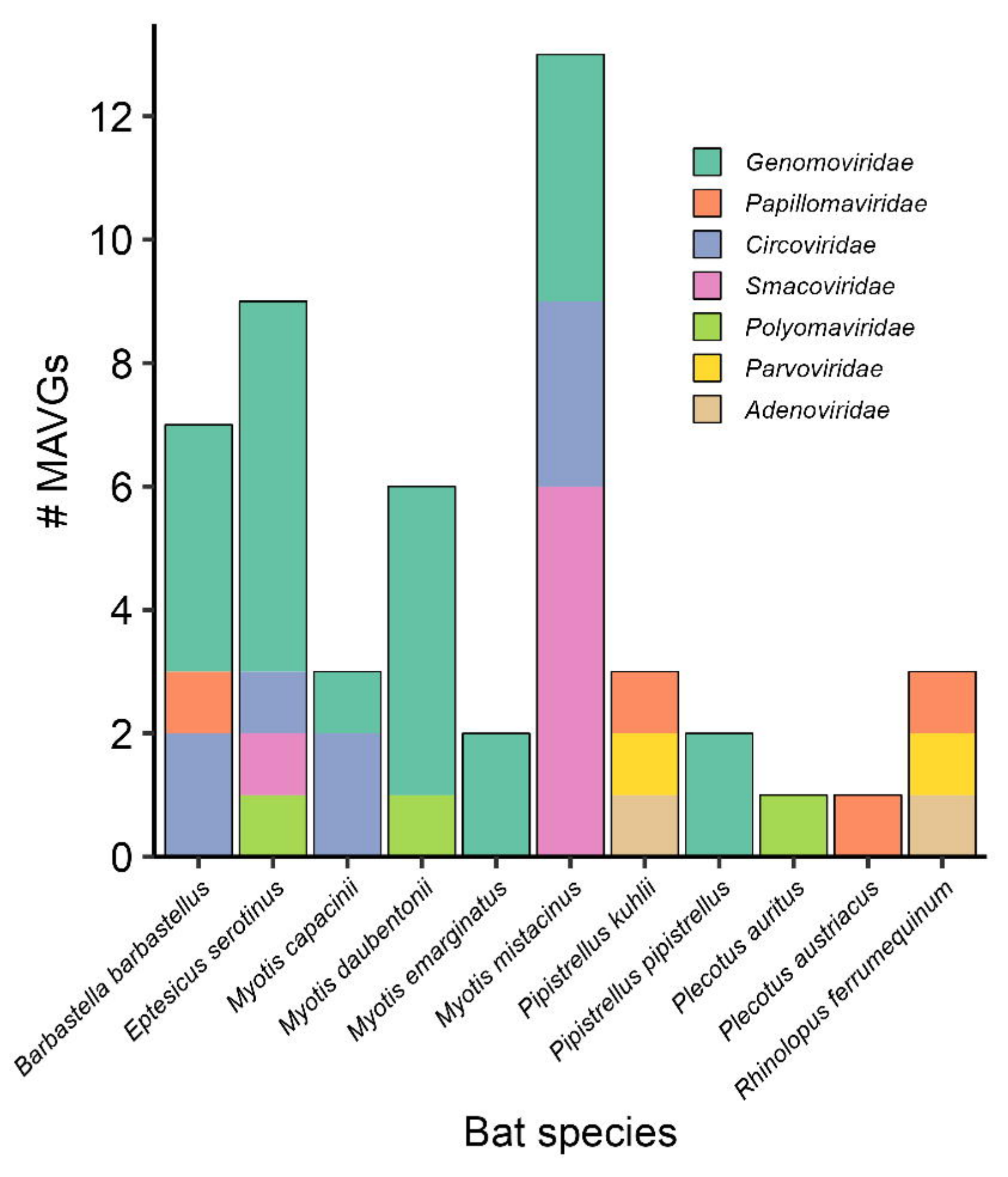
Supplementary Table S2. Primers used for viral sample confirmation by PCR.

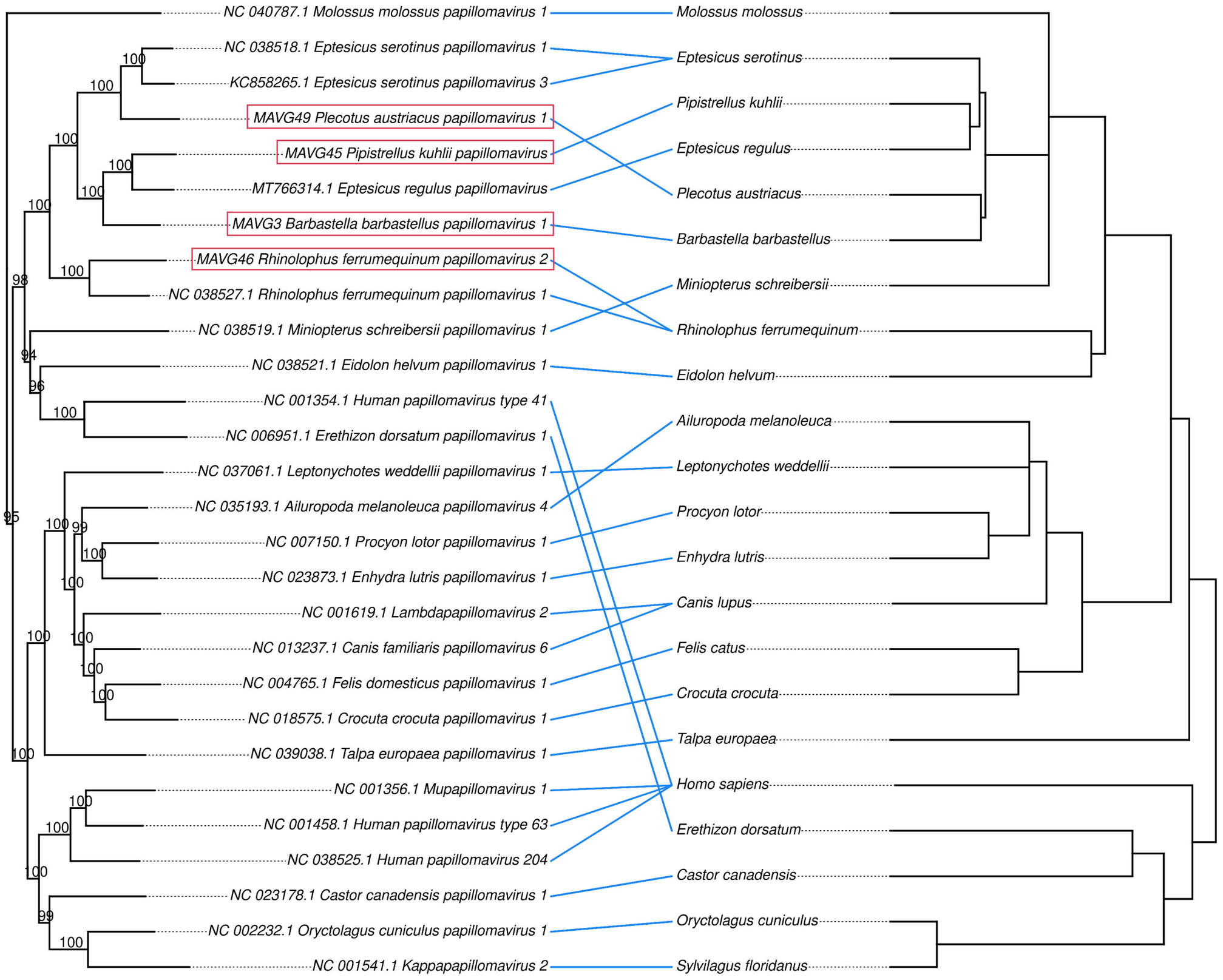
Supplementary Table S3. Illumina reads and number of viral contigs obtained.

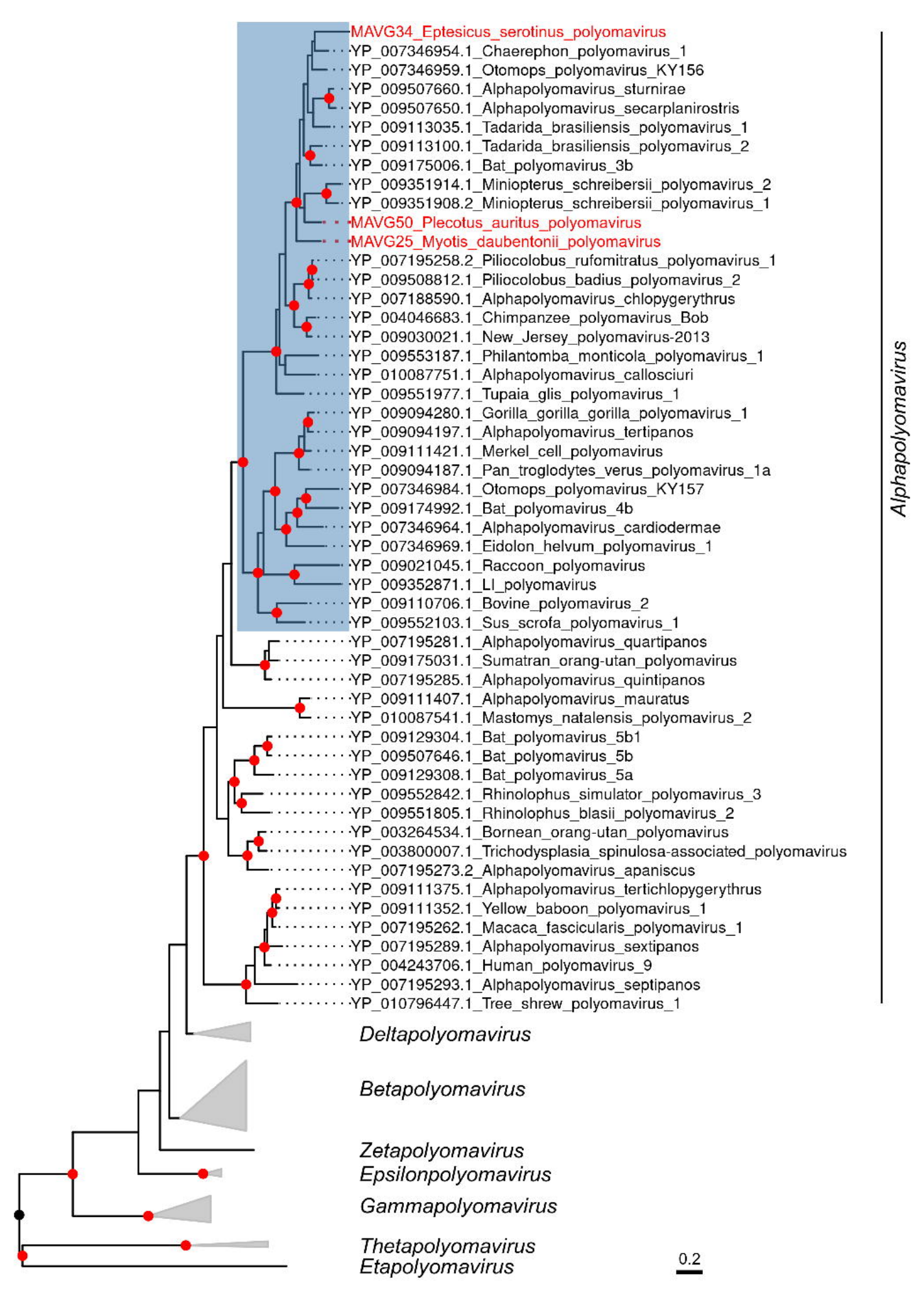
Supplementary Table S4. Descriptions, accession numbers and proposed names for the novel MAVGs.

Supplementary Table S5. Novel genomovirus species clusters.


Begonte (12)
Rábade (23)
Campoo-Los Valles (2)
Lugo (1)
Outeiro de Rei (28)
Navarredonda de la Rinconada (27)
Ojos Negros (1)
Espeja (3)
San Miguel del Robledo (7)
Castellón (7)
El Saler (4)
Fontanares (8)
Mula (1)
Ricote (6)
Fortuna (13)
Totana (4)
Torre Pacheco (19)
Pliego (15)
Cartagena (1)
Fuente Álamo (7)


12
10
8
6
4
2
0
MAVGs
Genomoviridae
Papillomaviridae
Circoviridae
Smacoviridae
Polyomaviridae
Parvoviridae
Adenoviridae
Barbastella barbastellus
Eptesicus serotinus
Myotis capacinii
Myotis daubentonii
Myotis emarginatus
Myotis mistacinus
Pipistrellus kuhlii
Pipistrellus pipistrellus
Plecotus auritus
Plecotus austriacus
Rhinolopus ferrumequinum
Bat species


NC 040787.1 Molossus molossus papillomavirus 1
NC 038518.1 Eptesicus serotinus papillomavirus 1
KC858265.1 Eptesicus serotinus papillomavirus 3
MAVG49 Plecotus austriacus papillomavirus 1
MAVG45 Pipistrellus kuhlii papillomavirus
MT766314.1 Eptesicus regulus papillomavirus
MAVG3 Barbastella barbastellus papillomavirus 1
MAVG46 Rhinolophus ferrumequinum papillomavirus 2
NC 038527.1 Rhinolophus ferrumequinum papillomavirus 1
NC 038519.1 Miniopterus schreibersii papillomavirus 1
NC 038521.1 Eidolon helvum papillomavirus 1
NC 001354.1 Human papillomavirus type 41
NC 006951.1 Erethizon dorsatum papillomavirus 1
NC 037061.1 Leptonychotes weddellii papillomavirus 1
NC 035193.1 Ailuropoda melanoleuca papillomavirus 4
NC 007150.1 Procyon lotor papillomavirus 1
NC 023873.1 Enhydra lutris papillomavirus 1
NC 001619.1 Lambdapapillomavirus 2
NC 013237.1 Canis familiaris papillomavirus 6
NC 004765.1 Felis domesticus papillomavirus 1
NC 018575.1 Crocuta crocuta papillomavirus 1
NC 039038.1 Talpa europaea papillomavirus 1
NC 001356.1 Mupapillomavirus 1
NC 001458.1 Human papillomavirus type 63
NC 038525.1 Human papillomavirus 204
NC 023178.1 Castor canadensis papillomavirus 1
NC 002232.1 Oryctolagus cuniculus papillomavirus 1
NC 001541.1 Kappapapillomavirus 2
Molossus molossus
Eptesicus serotinus
Pipistrellus kuhlii
Eptesicus regulus
Plecotus austriacus
Barbastella barbastellus
Miniopterus schreibersii
Rhinolophus ferrumequinum
Eidolon helvum
Ailuropoda melanoleuca
Leptonychotes weddellii
Procyon lotor
Enhydra lutris
Canis lupus
Felis catus
Crocuta crocuta
Talpa europaea
Homo sapiens
Erethizon dorsatum
Castor canadensis
Oryctolagus cuniculus
Sylvilagus floridanus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YP_009113035.1_Tadarida_brasiliensis_polyomavirus_1
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YP_009175006.1_Bat_polyomavirus_3b
YP_009351914.1_Miniopterus_schreibersii_polyomavirus_2
YP_009351908.2_Miniopterus_schreibersii_polyomavirus_1
MAVG50_Plecotus_auritus_polyomavirus
MAVG25_Myotis_daubentonii_polyomavirus
YP_007195258.2_Piliocolobus_rufomitratus_polyomavirus_1
YP_009508812.1_Piliocolobus_badius_polyomavirus_2
YP_007188590.1_Alphapolyomavirus_chlopygerythrus
YP_004046683.1_Chimpanzee_polyomavirus_Bob
YP_009030021.1_New_Jersey_polyomavirus-2013
YP_009553187.1_Philantomba_monticola_polyomavirus_1
YP_010087751.1_Alphapolyomavirus_callosciuri
YP_009551977.1_Tupaia_glis_polyomavirus_1
YP_009094280.1_Gorilla_gorilla_gorilla_polyomavirus_1
YP_009094197.1_Alphapolyomavirus_tertipanos
YP_009111421.1_Merkel_cell_polyomavirus
YP_009094187.1_Pan_troglodytes_verus_polyomavirus_1a
YP_007346984.1_Otomops_polyomavirus_KY157
YP_009174992.1_Bat_polyomavirus_4b
YP_007346964.1_Alphapolyomavirus_cardiodermae
YP_007346969.1_Eidolon_helvum_polyomavirus_1
YP_009021045.1_Raccoon_polyomavirus
YP_009352871.1_LI_polyomavirus
YP_009110706.1_Bovine_polyomavirus_2
YP_009552103.1_Sus_scrofa_polyomavirus_1
YP_007195281.1_Alphapolyomavirus_quartipanos
YP_009175031.1_Sumatran_orang-utan_polyomavirus
YP_007195285.1_Alphapolyomavirus_quintipanos
YP_009111407.1_Alphapolyomavirus_mauratus
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YP_009129304.1_Bat_polyomavirus_5b1
YP_009507646.1_Bat_polyomavirus_5b
YP_009129308.1_Bat_polyomavirus_5a
YP_009552842.1_Rhinolophus_simulator_polyomavirus_3
YP_009551805.1_Rhinolophus_blasii_polyomavirus_2
YP_003264534.1_Bornean_orang-utan_polyomavirus
YP_003800007.1_Trichodysplasia_spinulosa-associated_polyomavirus
YP_007195273.2_Alphapolyomavirus_apaniscus
YP_009111375.1_Alphapolyomavirus_tertichlopygerythrus
YP_009111352.1_Yellow_baboon_polyomavirus_1
YP_007195262.1_Macaca_fascicularis_polyomavirus_1
YP_007195289.1_Alphapolyomavirus_sextipanos
YP_004243706.1_Human_polyomavirus_9
YP_007195293.1_Alphapolyomavirus_septipanos
YP_010796447.1_Tree_shrew_polyomavirus_1
Alphapolyomavirus
Deltapolyomavirus
Betapolyomavirus
Zetapolyomavirus
Epsilonpolyomavirus
Gammapolyomavirus
Thetapolyomavirus
Etapolyomavirus
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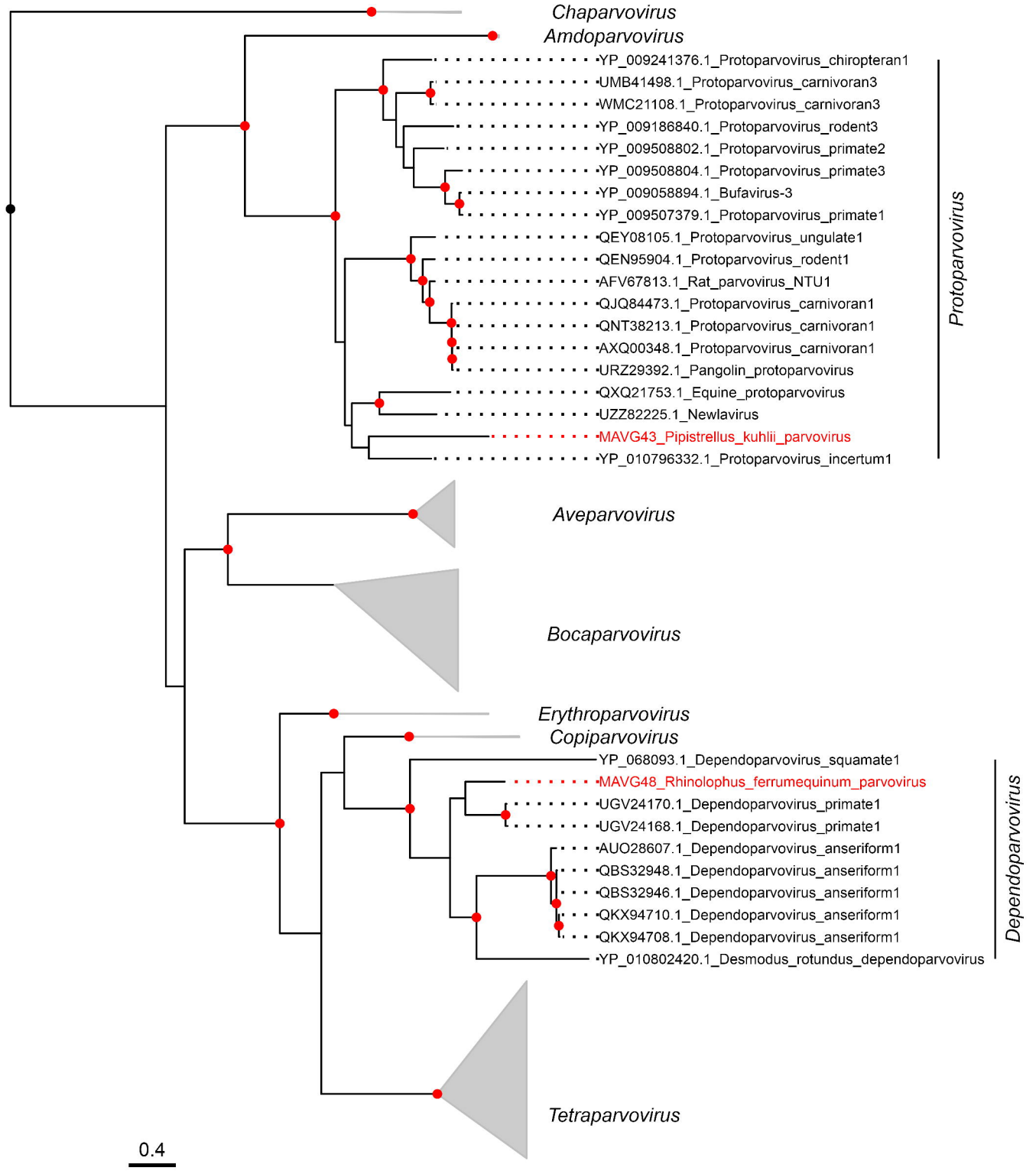
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YP_009508802.1_Protoparvovirus_primate2
YP_009508804.1_Protoparvovirus_primate3
YP_009058894.1_Bufavirus-3
YP_009507379.1_Protoparvovirus_primate1
QEY08105.1_Protoparvovirus_ungulate1
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AFV67813.1_Rat_parvovirus_NTU1
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AXQ00348.1_Protoparvovirus_carnivoran1
URZ29392.1_Pangolin_protoparvovirus
QXQ21753.1_Equine_protoparvovirus
UZZ82225.1_Newlavirus
MAVG43_Pipistrellus_kuhlii_parvovirus
YP_010796332.1_Protoparvovirus_incertum1
Protoparvovirus
Aveparvovirus
Bocaparvovirus
Erythroparvovirus
Copiparvovirus
YP_068093.1_Dependoparvovirus_squamate1
MAVG48_Rhinolophus_ferrumequinum_parvovirus
UGV24170.1_Dependoparvovirus_primate1
UGV24168.1_Dependoparvovirus_primate1
AUO28607.1_Dependoparvovirus_anseriform1
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Tetraparvovirus
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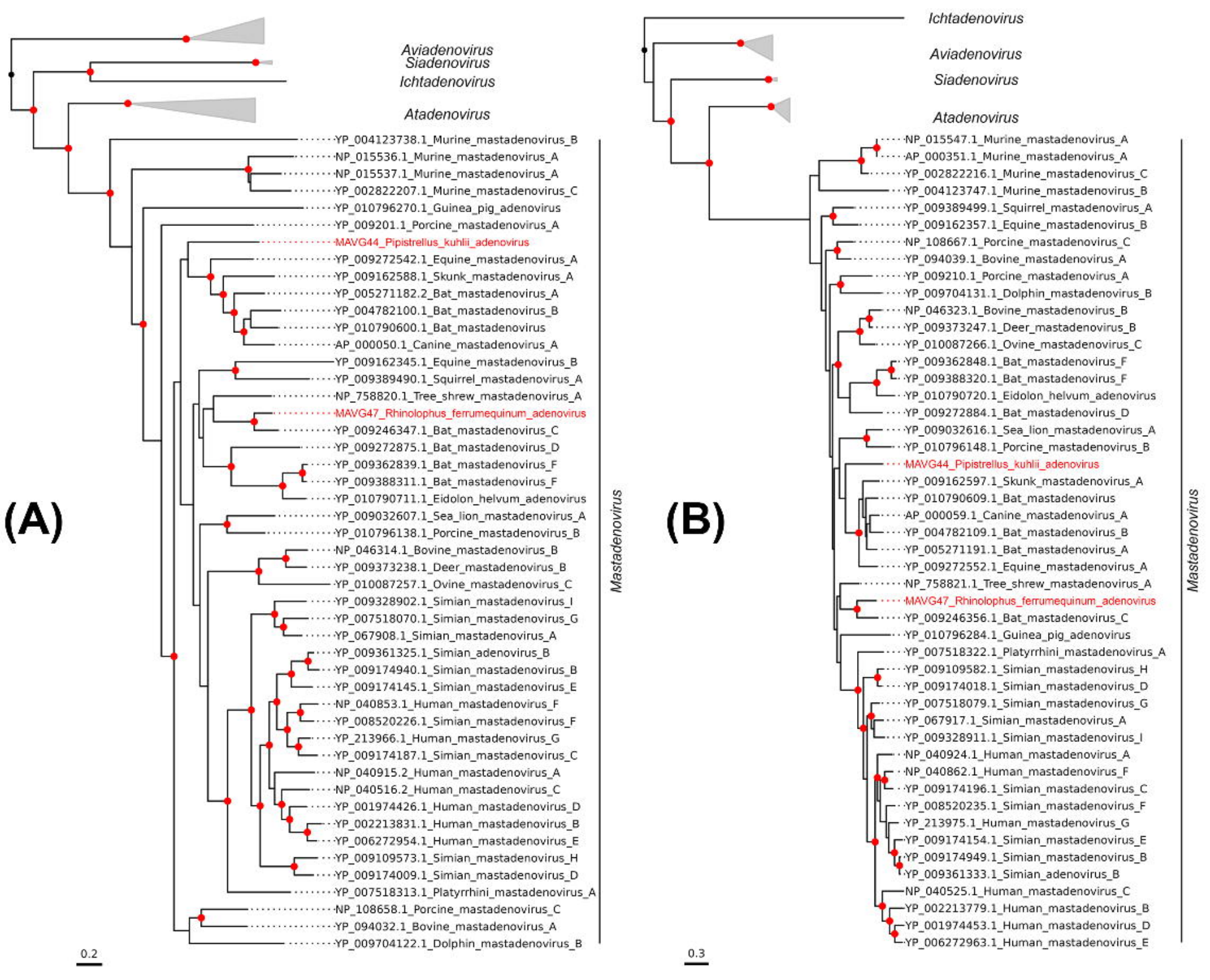
(A)
Aviadenovirus
Siadenovirus
Ichtadenovirus
Atadenovirus
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NP_015536.1_Murine_mastadenovirus_A
NP_015537.1_Murine_mastadenovirus_A
YP_002822207.1_Murine_mastadenovirus_C
YP_010796270.1_Guinea_pig_adenovirus
YP_009201.1_Porcine_mastadenovirus_A
MAVG44_Pipistrellus_kuhlii_adenovirus
YP_009272542.1_Equine_mastadenovirus_A
YP_009162588.1_Skunk_mastadenovirus_A
YP_005271182.2_Bat_mastadenovirus_A
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YP_010790600.1_Bat_mastadenovirus
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YP_009162345.1_Equine_mastadenovirus_B
YP_009389490.1_Squirrel_mastadenovirus_A
NP_758820.1_Tree_shrew_mastadenovirus_A
MAVG47_Rhinolophus_ferrumequinum_adenovirus
YP_009246347.1_Bat_mastadenovirus_C
YP_009272875.1_Bat_mastadenovirus_D
YP_009362839.1_Bat_mastadenovirus_F
YP_009388311.1_Bat_mastadenovirus_F
YP_010790711.1_Eidolon_helvum_adenovirus
YP_009032607.1_Sea_lion_mastadenovirus_A
YP_010796138.1_Porcine_mastadenovirus_B
NP_046314.1_Bovine_mastadenovirus_B
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YP_010087257.1_Ovine_mastadenovirus_C
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YP_067908.1_Simian_mastadenovirus_A
YP_009361325.1_Simian_adenovirus_B
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YP_009174145.1_Simian_mastadenovirus_E
NP_040853.1_Human_mastadenovirus_F
YP_008520226.1_Simian_mastadenovirus_F
YP_213966.1_Human_mastadenovirus_G
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YP_009109573.1_Simian_mastadenovirus_H
YP_009174009.1_Simian_mastadenovirus_D
YP_007518313.1_Platyrrhini_mastadenovirus_A
NP_108658.1_Porcine_mastadenovirus_C
YP_094032.1_Bovine_mastadenovirus_A
YP_009704122.1_Dolphin_mastadenovirus_B
Mastadenovirus
0.2
(B)
Ichtadenovirus
Aviadenovirus
Siadenovirus
Atadenovirus
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YP_004123747.1_Murine_mastadenovirus_B
YP_009389499.1_Squirrel_mastadenovirus_A
YP_009162357.1_Equine_mastadenovirus_B
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YP_094039.1_Bovine_mastadenovirus_A
YP_009210.1_Porcine_mastadenovirus_A
YP_009704131.1_Dolphin_mastadenovirus_B
NP_046323.1_Bovine_mastadenovirus_B
YP_009373247.1_Deer_mastadenovirus_B
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YP_009388320.1_Bat_mastadenovirus_F
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YP_009272884.1_Bat_mastadenovirus_D
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YP_010796148.1_Porcine_mastadenovirus_B
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AP_000059.1_Canine_mastadenovirus_A
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YP_009272552.1_Equine_mastadenovirus_A
NP_758821.1_Tree_shrew_mastadenovirus_A
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YP_010796284.1_Guinea_pig_adenovirus
YP_007518322.1_Platyrrhini_mastadenovirus_A
YP_009109582.1_Simian_mastadenovirus_H
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YP_067917.1_Simian_mastadenovirus_A
YP_009328911.1_Simian_mastadenovirus_I
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NP_040862.1_Human_mastadenovirus_F
YP_009174196.1_Simian_mastadenovirus_C
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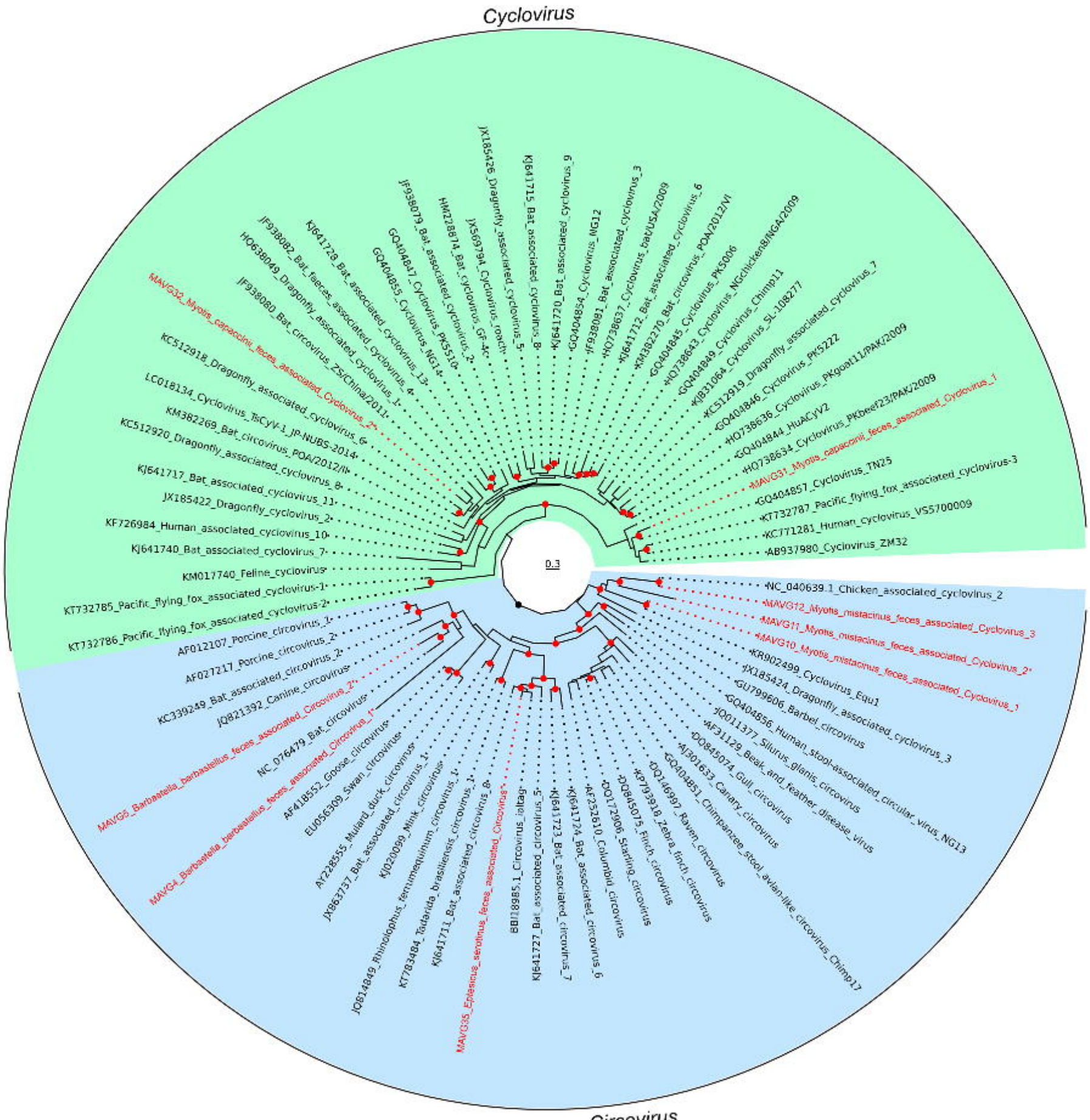
Cyclovirus
Circovirus
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JF938081_Bat_associated_cyclovirus_3
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KJ641712_Bat_associated_cyclovirus_6
KM382270_Bat_circovirus_POA/2012/VI
GQ404845_Cyclovirus_PK5006
HQ738643_Cyclovirus_NGchicken8/NGA/2009
GQ404849_Cyclovirus_Chimp11
KJ831064_Cyclovirus_SL-108277
KC512919_Dragonfly_associated_cyclovirus_7
GQ404846_Cyclovirus_PK5222
HQ738636_Cyclovirus_PKgoat11/PAK/2009
GQ404844_HuACyV2
HQ738634_Cyclovirus_PKbeef23/PAK/2009
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KT732787_Pacific_flying_fox_associated_cyclovirus-3
KC771281_Human_cyclovirus_VS5700009
AB937980_Cyclovirus_ZM32
JF938082_Bat_faeces_associated_Cyclovirus_4
KJ641728_Bat_associated_Cyclovirus_13
GQ404847_Cyclovirus_NG14
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JF938079_Bat_associated_Cyclovirus_2
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JF938080_Bat_circovirus_ZS/China/2011
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KC512918_Dragonfly_associated_cyclovirus_6
LC018134_Cyclovirus_TsCyV-1_JP-NUBS-2014
KM382269_Bat_circovirus_POA/2012/IV
KC512920_Dragonfly_associated_cyclovirus_8
KJ641717_Bat_associated_cyclovirus_11
JX185422_Dragonfly_cyclovirus_2
KF726984_Human_associated_cyclovirus_10
KJ641740_Bat_associated_cyclovirus_7
KM017740_Feline_cyclovirus
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KT732786_Pacific_flying_fox_associated_cyclovirus-2
AF012107_Porcine_circovirus_1
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KC339249_Bat_associated_circovirus_2
JQ821392_Canine_circovirus
MAVG6_Barbastella_barbastellus_feces_associated_Circovirus_2
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KJ641724_Bat_associated_circovirus_6
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DQ172006_Starling_circovirus
DQ845075_Finch_circovirus
KP793918_Zebra_finch_circovirus
GQ146997_Raven_circovirus
GQ404851_Chimpanzee_stool_avian-like_circovirus_Chimp17
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AJ301633_Canary_circovirus
AF311129_Beak_and_feather_disease_virus
JQ011377_Silurus_glanis_circovirus
GQ404856_Human_stool-associated_circular_virus_NG13
GU799606_Barbel_circovirus
JX185424_Dragonfly_associated_cyclovirus_3
KR902499_Cyclovirus_Equ1
MAVG10_Myotis_mistacinus_feces_associated_Cyclovirus_1
MAVG11_Myotis_mistacinus_feces_associated_Cyclovirus_2
MAVG12_Myotis_mistacinus_feces_associated_Cyclovirus_3
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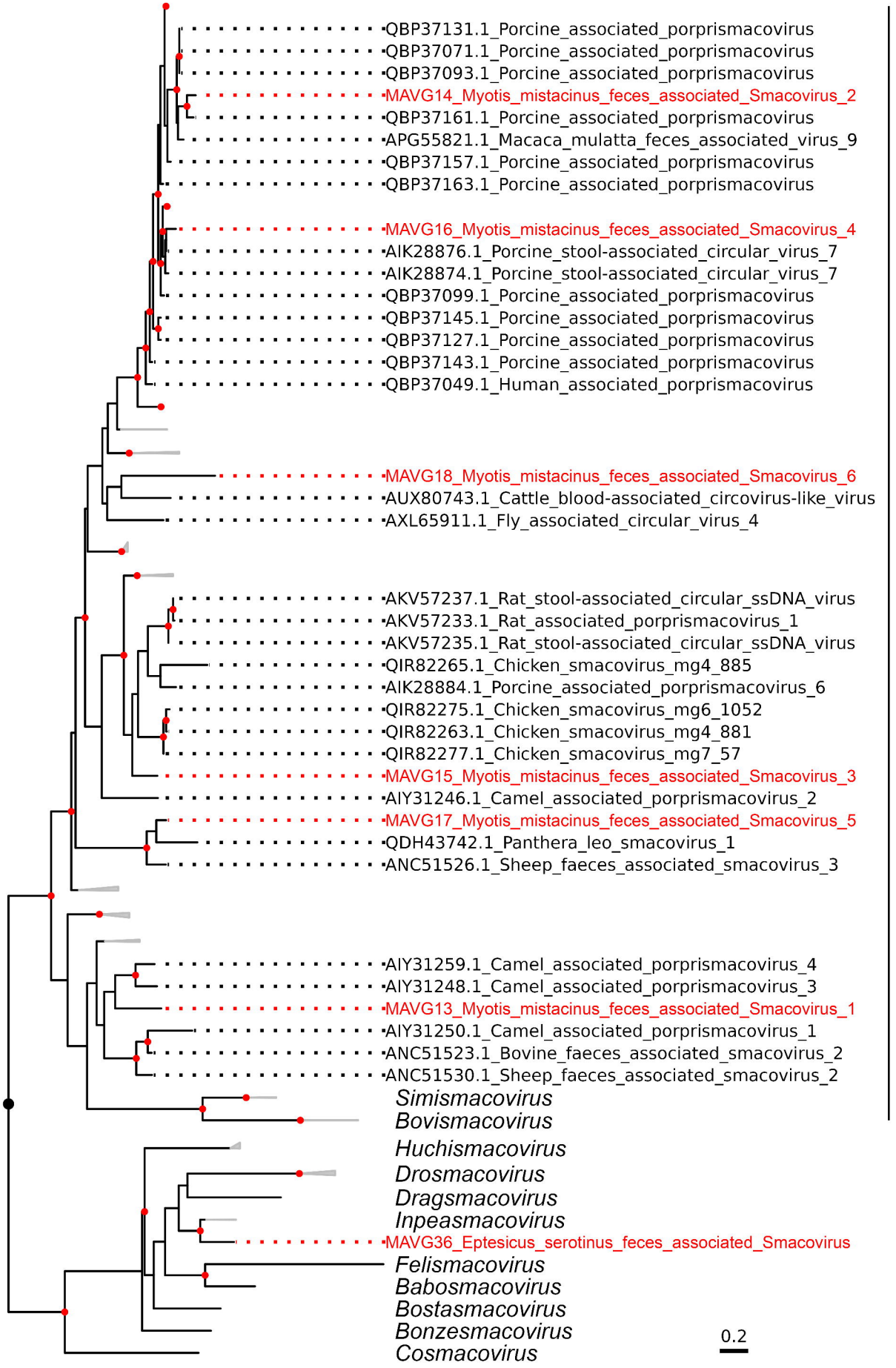
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QBP37163.1_Porcine_associated_porprismacovirus
MAVG16_Myotis_mistacinus_feces_associated_Smacovirus_4
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QBP37143.1_Porcine_associated_porprismacovirus
QBP37049.1_Human_associated_porprismacovirus
MAVG18_Myotis_mistacinus_feces_associated_Smacovirus_6
AUX80743.1_Cattle_blood-associated_circovirus-like_virus
AXL65911.1_Fly_associated_circular_virus_4
AKV57237.1_Rat_stool-associated_circular_ssDNA_virus
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MAVG15_Myotis_mistacinus_feces_associated_Smacovirus_3
AIY31246.1_Camel_associated_porprismacovirus_2
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Porprismacovirus
Simismacovirus
Bovismacovirus
Huchismacovirus
Drosmacovirus
Dragsmacovirus
Inpeasmacovirus
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Bostasmacovirus
Bonzesmacovirus
Cosmacovirus
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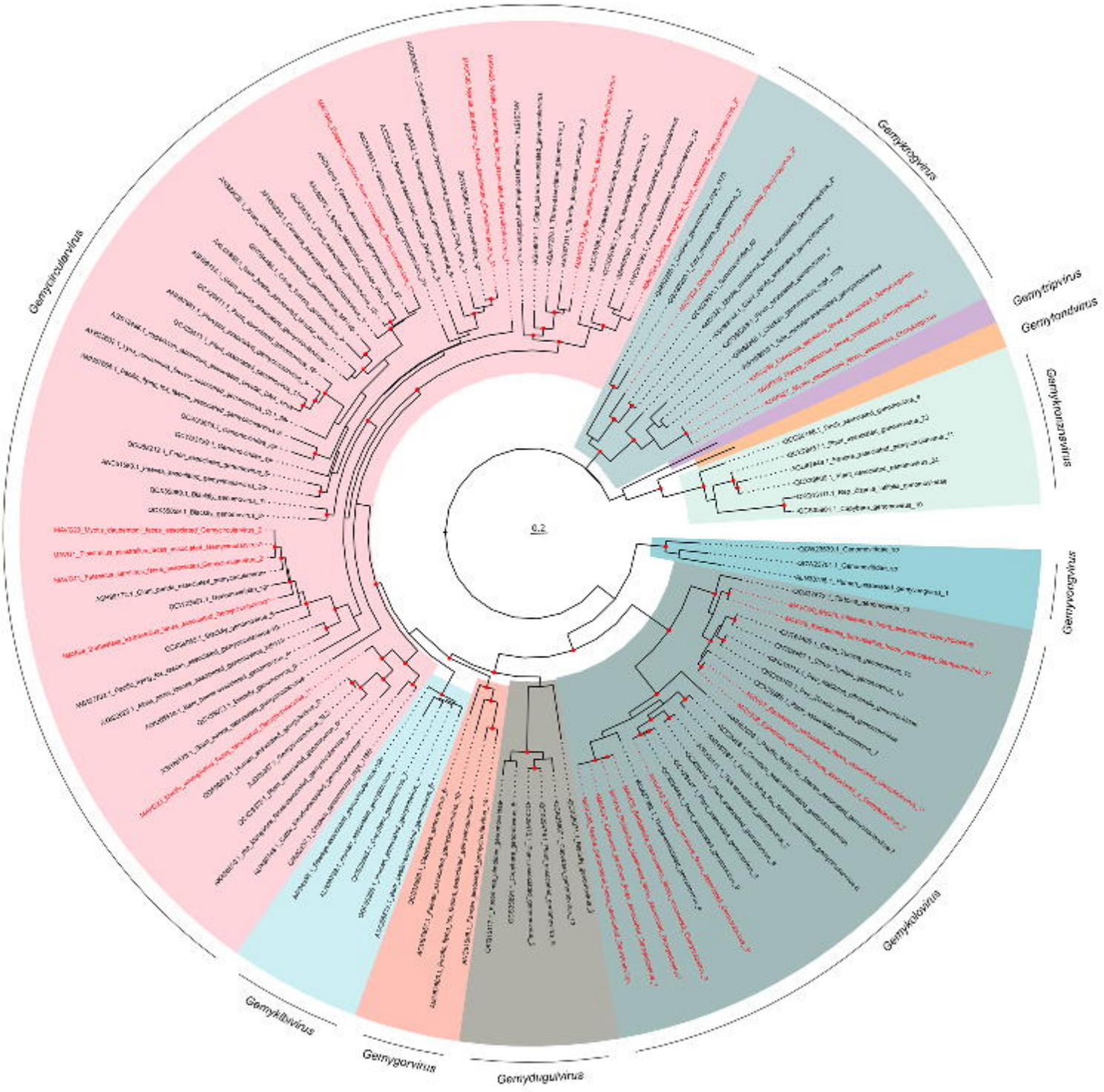
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