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DNA polymerase sequences of New World monkey cytomegaloviruses: another molecular marker to infer Platyrrhini systematics

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ABSTRACT

Over the past few decades, a large number of studies have identified herpesvirus sequences from many mammalian species around the world. Among the different non-human primate species tested so far for cytomegaloviruses, only a few were from the New World. Seeking to identify cytomegalovirus homologues in New World monkeys (NWMs), we carried out molecular screening of 244 blood DNA samples from 20 NWM species from Central and South America. Our aim was to reach a better understanding of their evolutionary processes within the Platyrrhini parvorder. Using polymerase chain reaction amplification with degenerate consensus primers targeting highly conserved amino acid motifs of the herpesvirus *DNA polymerase* gene, we characterized novel viral sequences from 12 species belonging to seven genera representative of the three NWM families. BLAST searches, pairwise nucleotide and amino acid sequence comparisons, as well as phylogenetic analyses confirmed that they all belonged to the *Cytomegalovirus* genus. Previously determined host taxa allowed us to demonstrate a good correlation between the distinct monophyletic clades of viruses with those of the infected primates at the genus level. In addition, the evolutionary branching points that separate NWM CMVs were congruent with the divergence dates of their hosts at the genus level. These results significantly expand our knowledge of the host range of this viral genus and strongly support the co-speciation between these viruses and their hosts. In this respect, we propose that NWM CMV *DNA polymerase* sequences could serve as a reliable molecular marker to infer Platyrrhini phylogenetics.

IMPORTANCE

Investigating evolutionary processes between viruses and non-human primates has led to the discovery of a high number of herpesviruses. No study published so far on primate cytomegaloviruses has extensively studied New World monkeys (NWMs) at the subspecies, species, genus and family levels. The present study sought to identify cytomegalovirus homologues in NWMs and decipher their evolutionary relationships. This led us to characterize novel viruses in 12 of the 20 primate species tested representative of the three NWM families. The identification of distinct viruses in these primates not only significantly expands our knowledge of the host range of this viral genus, but has also shed light on their evolutionary history. Phylogenetic analyses and molecular dating of the sequences obtained support a virus–host coevolution.

INTRODUCTION

New World monkeys of tropical forests from Central to South America belong to the Platyrrhini parvorder (1). They first appeared in the Neotropics in the late Eocene or early Oligocene and have subsequently evolved into a broad diversity of families, subfamilies and genera (**Figure 1**) (2). To shed light on their phylogeny and evolution, NWMs have been extensively studied through morphological, bio-geographical, behavioral and molecular data (2-16). Over the last few decades, contrasting hypotheses have been proposed, presumably due to different markers and the presence of polymorphisms in the features considered. Agreement on the main clades of NWMs has been reached using different approaches, revealing a unique phylogenetic arrangement of Platyrrhini with three monophyletic families: Pitheciidae, Atelidae and Cebidae (**Table 1**, **Figure 1**) (3, 4, 10, 11, 13-15). Nevertheless, the relationships between them continue to be debated. Through the analysis of intergeneric and intrageneric relationships, intrafamily relationships have also been studied in depth. By incorporating all the available data, major advances have been made and many taxonomic controversies have been clarified (5). Therefore, the Pitheciidae family is composed of *Callicebus*, *Pithecia*, *Chiropotes* and *Cacajao*, the Atelidae family of *Alouatta*, *Ateles*, *Brachyteles* and *Lagothrix* while the Cebidae family is divided into *Cebuella*, *Mico*, *Callithrix*, *Callimico*, *Saguinus*, *Leontopithecus*, *Samiri*, *Cebus*, *Sapajus* and *Aotus* genera. However, relationships between or within some subfamilies and/or genera remain under discussion. Among Cebidae, the phylogenetic position of the Aotinae subfamily remains unclear (14). Indeed, molecular data did not allow determining if Aotinae is a sister clade of Callitrichinae or if, alternatively, Aotinae, Saimiriinae and Cebinae are sisters to Callitrichinae (3, 11, 13-15). Moreover, the number of platyrrhine genera is also still under discussion, such as the division of *Cebus* into the *Sapajus* (tufted capuchins) and *Cebus* (untufted capuchins) genera (3, 16-18). As a result, neither the diversity nor the taxonomy of NWMs are fully known. To

appreciate the details of Platyrrhini's evolution, much work still needs to be done at various taxonomic levels.

Viruses of the genus *Cytomegalovirus* belong to the *Betaherpesvirinae* subfamily within the *Herpesviridae* family, order *Herpesvirales* (19). Eight cytomegaloviruses are recognized as species by the International Committee for Taxonomy of Viruses (ICTV) according to the latest master species list (MSL# 32) released on March 12, 2018 (https://talk.ictvonline.org/files/master-species-lists/m/msl/7185). *Human betaherpesvirus 5* (HHV5), commonly referred to in the literature as human cytomegalovirus (HCMV), is the cytomegalovirus (CMV) type species. So far, cytomegaloviruses have been characterized only from primates. Since the initial description of cytomegalovirus in African green monkeys in 1957, whose current species name is *Cercopithecine betaherpesvirus 5* (CeHV5), natural infections by such viruses have been described in several Old World monkey (OWM) species, including baboons, macaques, colobuses, chimpanzees, gorillas and others (20-31). In contrast, cytomegaloviruses of New World monkeys (NWMs) are represented by only three viral entities from *Aotus trivirgatus* (northern owl monkey), *Saimiri sciureus* (common squirrel monkey) and *Cebus* spp. (capuchin) animals, despite the wide diversity of platyrrhines (32-34). It is presumed that all primate species harbor CMVs following a co-speciation process, but data supporting this assumption are scarce. The most extensive analyses of primate CMVs conducted to date are those of Leendertz *et al.* (26) and Anoh *et al.* (30). These studies have demonstrated, through phylogenetic analyses, a species-specific distribution of these viruses. This species specificity indicates a long-term coevolution of CMVs with their natural hosts. The identification of two clades, each composed of chimpanzee and gorilla CMVs, suggests that they have coevolved

following a horizontal transmission event between these great apes millions of years ago (26). Nevertheless, interspecies transmissions in the wild are rare events (26, 28, 30, 31).

With the exception of the three above-mentioned CMVs of NWMs, there has been little prior organized effort to discover cytomegaloviruses in neotropical primates. The number of NWM species tested to date therefore accounts for only a tiny part of their diversity. We thought that additional investigations on a larger number of species were required. We therefore addressed the possible presence of CMVs in different NWM species from which we previously partially characterized Epstein-Barr-like viruses (35, 36). The purpose was to gain greater insight into the distribution and diversity of CMVs infecting the Platyrrhine primates. Furthermore, based on the coevolution observed between OWMs and their specific CMVs, we wished to determine whether NWM CMV sequences could help decipher evolutionary relationships of their host species (26, 30). Given that multiple molecular markers of mitochondrial and nuclear DNAs are available, host species can be characterized along with their viruses, allowing progress to be made on their respective pattern of diversification. Here, we report finding sequences of cytomegaloviruses in different NWM species and achieve a better understanding of the evolutionary processes between these viruses and their Platyrrhini hosts.

RESULTS

To look for the presence of CMV-like viruses in our collection of NWMs, we attempted to amplify a fragment of the highly conserved herpesvirus *DNA polymerase* gene from the PBMC DNA of each wild-caught primate using the PCR conditions as described previously (27, 35). A total of 244 samples from 20 different species of the three NWM families were tested (**Table 1**). DNA samples from 12 species scored positive after the nested PCRs (nPCRs) (**Table 1**). No

primate belonging to the *Saguinus* and *Lagothrix* genera scored positive. Indeed, no amplification was observed in any samples from the three tamarin species (*Saguinus midas*, *S. labiatus* and *S. oedipus*) and the four woolly monkey subspecies (*Lagothrix lagotricha cana*, *L. l. lagotricha*, *L. l. lugens* and *L. l. poeppigii*). We then used different pairs of consensus-degenerate and specific PCR primers to obtain longer sequences of the *DNA polymerase* gene from each positive animal (**Table 2, Figure 2**). The concatenated nucleotide sequences generated are between 448 and 2,026 bp in size depending on the viral strain (**Table 1**).

BLAST searches demonstrated that all sequences identified belonged to the *Cytomegalovirus* genus and revealed the presence of 12 distinct sequences. Four sequences were identified twice in the *Saimiri boliviensis boliviensis*, *Aotus nancymaae*, *Pithecia pithecia* and *Alouatta seniculus* species while the sequence in the *Alouatta macconnelli* was identified in five individuals (**Table 1**). Virus names and abbreviations were given to the 12 distinct viruses (**Table 1**): the viruses were named after the host species (for *Saimiri*, after the subspecies) followed by three upper case letters corresponding to the viral genus (CMV for *Cytomegalovirus*) to which they were then assigned the Arabic numeral 1, as has already been done by us and others (26, 35).

To obtain a full vision of the genetic diversity of these new CMV sequences, pairwise sequence comparisons were made on the 447-bp/149-aa fragments of the *DNA polymerase* gene common to all primate CMVs. All 12 sequences obtained differed from each other at the nucleotide level. Sequences that were identified in different specimens of the same primate species, e.g., AnanCMV1, AsenCMV1, AmacCMV1, SbolCMV1 and PpitCMV1, were 100% identical with the exception of the two PpitCMV1 sequences showing 99.6% nucleotide identity (**Table 3**). For clarity, comparison of the percentage of identity between the different newly

identified NWM CMVs have been reported by grouping viral sequences at the host genus level (**Table 3**). Overall, the new sequences exhibited among themselves and the other available NWM CMV sequences from 71.1% (AnanCMV1 *vs.* ApanCMV1) to 99.6% (CalbCMV1 *vs.* CebHV1 or *Cebus* sp. herpesvirus) nucleotide identity and from 79.6% (CcapCMV1 *vs.* ApanCMV1 and AnanCMV1 *vs.* ApalCMV1) to 100% (AmacCMV1 *vs.* AsenCMV1 as well as SbolCMV1 *vs.* SalbCMV1) amino acid identity (**Table 3**). Viruses infecting NWMs of the same genus presented more than 92% of nucleotide and amino acid identities (**Table 3**). Comparison between CMVs of different NWM genera ranged from 71.1% (*Aotus* CMVs *vs. Ateles* CMVs) to 88.7% (*Sapajus* CMVs *vs. Cebus* CMVs) at the nucleotide level and from 79.6% between *Aotus* and *Alouatta* CMVs and *Cebus* and *Ateles* CMVs to 99.3% between *Sapajus* and *Cebus* CMVs at the amino acid level. NWM CMV sequences exhibited 59.8% (*Cebus* CMVs *vs. Macaca* CMVs) to 72.7% (*Aotus* CMVs *vs. Colobus* CMVs) nucleotide sequence identities and 61.9 to 68.7% amino acid sequence identities with OWM CMVs. The level of nucleotide and amino acid sequence identity with CMVs of Hominidae, except HHV5, ranged from 61 to 72% and from 66 to 72.8%, respectively. Identities with HHV5 ranged from 61.6% (*Alouatta* CMVs) to 70.9% (*Aotus* CMVs) at the nucleotide level and from 66% (*Alouatta* CMVs) to 72.8% (*Aotus* CMVs) at the amino acid level.

All phylogenetic analyses, performed on nucleotide or amino acid sequences between the newly characterized CMV sequences and those of other primate CMVs available in the databases clearly placed the new sequences in a monophyletic lineage of NWM viruses in the *Cytomegalovirus* genus. The phylogenetic analysis presented in **Figure 3** is based on amino acid sequences. The NWM CMV lineage diverged from the OWM CMV lineage with a posterior probability value of 1. Remarkably, considering the OWM CMV lineage, the phylogenetic tree

formed two major monophyletic groups consisting of Hominoidea and Cercopithecoidea viruses, respectively. Within the Cercopithecoidea, the *Colobus* CMV sequences are the basal taxa with the formation of additional clades, comprising Asian *Macaca*, *Cercopithecus*/*Chlorocebus* and *Papio/Mandrillus/Cercocebus* taxa.

Considering NWM CMVs, analyses demonstrated the existence of five distinct lineages supported by high posterior probability values. The phylogenetic relationships between the different NWM CMVs were correlated with the families and genera to which the infected primates belong. The only exception was the hierarchical branching order of the *Aotus*/*Saimiri*/*Cebus* genera within the Cebidae, which was not supported. Thus, viruses of *Aotus* (AoHV1, AvocCMV1 and AnanCMV1) all grouped together in a monophyletic clade as did those of *Saimiri* (SaHV4, SscHV, SalbCMV1 and SbolCMV1), *Cebus/Sapajus* (CebHV1, CebusHV, CalbCMV1, CcapCMV1 and SapeCMV1), *Alouatta* (ApalCMV1, AmacCMV1 and AsenCMV1) and *Pithecia* (PpitCMV1). Furthermore, viruses from *Alouatta* species were related to ApanCMV1 from *Ateles* in a monophyletic clade of viruses infecting Atelidae monkeys with a posterior probability of 1, while those of *Saimiri*, *Aotus* and *Cebus/Sapajus* belonged to a monophyletic clade of Cebidae, which was supported by a posterior probability of 0.91.

To explore the cospeciation hypothesis, a time calibration analysis was performed on our data set. Cytomegaloviruses identified in NWMs diverged from those of OWMs at about 32.45 MYA (95% HPD: 17.76–52.33) (**Table 4**). In the New World clade, three major groups were identified corresponding to viruses hosted by (i) Pitheciidae, (ii) Atelidae and (iii) Cebidae. The Pitheciidae viruses diverged from those of the two other groups 22.33 MYA (95% HPD: 16.25–28.21) and intraspecific divergence of PpitCMV1 occurred 1.15 MYA. The divergence between

Atelidae and Cebidae viruses is estimated at 17.16 MYA (95% HPD: 10.29–24.25). Within the group of Atelidae viruses, the divergence between ApanCMV1 identified in *Ateles paniscus* with those identified in *Alouatta* spp. (ApalCMV1, AmacCMV1 and AsenCMV1) is estimated at 8.29 MYA (95% HPD: 2.57–14.82). Viruses identified in the three different *Alouatta* species (*palliata*, *macconnelli* and *seniculus*) diverged at 3.11 MYA (95% HPD: 0.47–6.82) (ApalCMV1 *vs.* AmacCMV1 /AsenCMV1) while AmacCMV1 diverged from AsenCMV1 1.33 MYA (95% HPD: 0.02–3.45). Within the Cebidae group, the divergence between viruses of Aotinae, Cebinae and Saimiriinae occurred around 11.54 MYA (95% HPD: 5.82–17.89). Within the group of cytomegaloviruses hosted by the different *Aotus* species, AoHV1 identified in *A. trivirgatus* diverged from the others about 3.98 MYA (95% HPD: 0.8–8.25) while AnanCMV1 diverged from AvocCMV1 2.15 MYA (95% HPD: 0.2–4.88). Within the group of viruses identified in the Cebinae, CcapCMV1 from *C. capucinus* diverged from the others 3.26 MYA (95% HPD: 0.59–6.94). SapCMV1 identified in *Sapajus apella* diverged from the other viruses detected in Cebus (*albifrons* and *Cebus* sp.) 2.09 MYA (0.32–4.68). Finally, within the group of viruses identified in the *Saimiri* genus, SsciCMV1/SaHV4 detected in *S. sciureus* diverged from those hosted by *S. boliviensis* and *S. albigena*, SbolCMV1 and SalbCMV1, 2.68 MYA (95% HPD: 0.28–6.14).

DISCUSSION

This study is the largest conducted to date to molecularly characterize CMVs in NWMs in terms of species diversity. It has partially characterized 12 cytomegaloviruses from 12 distinct species belonging to seven genera and three NWM families. BLAST searches of the *Cytomegalovirus* sequences identified further revealed that all but one were new viral sequences close to, but distinct from, already published CMV sequences from *Aotus trivirgatus*, *Saimiri sciureus* and *Cebus* sp. The only exception was the viral sequence from *C. albifrons* showing 99.6% identity at

222 the nucleotide level to CebusHV (AF292067) and CebHV1 (JQ264772), both identified from
223 unspecified *Cebus* spp. These three viral sequences were therefore considered to correspond to
224 the same viral species. In addition, the newly identified viral sequences are completely host-
225 specific, with no identification of cross-species transmission in our sample. Observations on
226 sequence comparisons, phylogenetic analysis and host-specificity of the sequences reported in
227 this paper are close to species demarcation criteria outlined in the 9th ICTV report for formal
228 recognition of new herpesvirus species (https://talk.ictvonline.org/ictv-reports/ictv_9th_report/)
229 (37).
230
231 By refining the degeneracy of the PCR primers used to screen the sample collection, we
232 were able to specifically target and identify CMV sequences, even though some of the primates
233 tested were coinfected with lymphocryptoviruses (35). Indeed, we formerly identified 17 EBV-
234 related viruses from 15 NWM species belonging to seven genera and three families from the
235 same collection of samples (35, 36). These new combinations of screening primers are therefore
236 good molecular tools to be used for future studies. Nevertheless, on the 20 NWM species tested,
237 we did not characterize any CMV sequence from our collection of *Saguinus* spp. and *Lagothrix*
238 spp. samples. Considering the relatively small sampling size of most species belonging to these
239 two genera, with the exception of *Saguinus midas*, it is conceivable that we missed a CMV-like
240 virus from them. Nevertheless, for the other primate species tested, the sampling size was
241 equivalent or even smaller and we identified CMV sequences for almost all of them. More
242 strikingly, despite the large sample size of *Saguinus midas* screened (54 individuals) and the
243 different PCR approaches used (different combinations of primers with different levels of
244 degeneracy and PCR cycling conditions), no PCR product was identified. The negativity of the
245 *Saguinus* and *Lagothrix* genera for CMV-related viruses can be explained by a lack of primer

246 matching or by a loss of CMV viruses during evolution within these genera. Likewise, in our
247 former studies of EBV-related sequences, we were unsuccessful in amplifying EBV sequences
248 from individuals of the *Aotus* and *Alouatta* genera (35, 36). Taken together, these results
249 highlight the need for more in-depth analyses of a representative sample from these and other
250 species of these genera to clarify this point. Moreover, for part of the positive samples, we were
251 unsuccessful in generating longer sequences of the *DNA polymerase* gene. Whether this is due to
252 the low quality/low amount of the remaining DNA, a low viral load, or reflects technical
253 difficulties, *i.e.* an inadequate level of degeneracy of the primers designed for some of these
254 viruses, is not clear. Nonetheless, sequence data generated here were sufficient to gain insight on
255 their genetic relationships.
256
257 Pairwise nucleotide and amino acid sequence comparisons demonstrated that the viral
258 sequences analyzed present different levels of genetic diversity among them (**Table 3**); the
259 smallest divergences were detected when viral sequences from primates belonging to the same
260 genus were analyzed. Phylogenetic analyses showed that CMV sequences are grouped according
261 to the primate genus from which they were detected. Thereafter, the phylogenetic clustering and
262 diversification follow those proposed for NWM species, corroborating the hypothesis of joint
263 evolution of the viruses with the speciation of their hosts (3, 5). Comparatively, analyses of
264 NWM EBV sequences have fallen short of achieving a completely resolved phylogeny (35).
265 While a clear co-speciation can be seen in the terminal branchings within major lineages
266 according to the primate subfamilies, the phylogenetic relationships between them are not
267 concordant with the current interpretations of their hosts' pattern of diversification at the family
268 level. In addition, for OWMs, there is a similar incongruence between the *Lymphocryptovirus*
269 phylogeny and that of the corresponding host lineages (38, 39). One can therefore argue that,

within the *Herpesviridae* family, *DNA polymerase* sequences from viruses of the *Cytomegalovirus* genus are a better molecular marker than those of the *Lymphocryptovirus* genus to test hypotheses of herpesvirus/primate co-evolution. On the basis of the available data, our analysis nevertheless has two limitations regarding viruses of Cebidae that do not perfectly reflect current interpretations of their hosts' diversification pattern. While viral sequences of Cebidae segregate into three well-supported clades, each corresponding to the host genus from which they have been identified, *i.e. Cebus*/*Sapajus*, *Saimiri* and *Aotus*, the relationships between the three clades are not phylogenetically supported (**Figure 3**). The second limitation concerns SapeCMV1, identified from *Sapajus apella*, which, phylogenetically, falls within the group of *Cebus* viruses (**Figure 3**). Nevertheless, pairwise sequence comparison of SapeCMV1 with the viral sequences identified from *Cebus* spp. shows that the nucleotide divergence of SapeCMV1 is over the maximum 8% observed for viral sequences identified from NWM species of the same genus (**Table 3**). These combined results (on SapeCMV1 and the other *Cebus* viruses) do not, for the moment, make it possible to confidently separate the *Cebus* genus into two genera as observed on analyses of *Alu* elements and on phylogenomics (3, 17). However, our virus results agree quite well with the mitogenomics findings obtained in recent studies, where *Sapajus* is a taxon within *Cebus* (18). These limits should be resolved by screening an extensive taxon sampling of the different *Sapajus* spp. as well as of Callithrichinae for the presence of cytomegaloviruses.

Finally, these data support virus–host coevolution in terms of branching order as well as divergence time. Indeed, for each NWM genus tested, the estimated timing of diversification of viruses is in agreement with host sequence divergence date estimates from previously published studies (**Table 4**) (3, 10, 15). Nevertheless, dates obtained at superior taxonomic levels are more

recent than those based on different types of data sets or models. These discrepancies can be attributed to the fact that a majority of NWM taxa remain to be tested and that no CMV sequence is available for numerous genera. This therefore limits the significance of our estimates for the major primate lineages for the moment and emphasizes the need for further studies.

Here, we conclusively expand our knowledge of the viral diversity, distribution and evolutionary relationships of NWM CMVs. Even if the evolutionary history of these viruses is not fully resolved, and in spite of the limitations mentioned above, these results strongly support the co-evolution hypothesis of these new viruses with their hosts. In light of these data, we propose that CMV *DNA polymerase* sequences could serve as a genetic marker to define the evolutionary links of their host species. Indeed, despite the number of studies conducted over the past few decades and the fast-growing number of host DNA sequence data sets, an unifying consensus of the evolutionary hierarchy of NWMs has not been fully reached, partly because not all phylogenies from these data sets agree but also due to a large proportion of missing data for some taxa (2-15). The search for and identification of CMV *DNA polymerase* sequences therefore seems to be an alternative to help solve this issue. Given the high prevalence rate of CMVs in wild primates, their spread through close contact with infectious bodily fluids and their persistence for the lifelong of the host, cytomegalovirus sequences, if present, should be easily obtained through our PCR approach (20, 21, 40-42). Considering the number of all presently known NWM species, we only tested here a fraction of their diversity. This suggests that a great number of cytomegaloviruses remains to be identified in this important group of primates. These results argue for a wider and more systematic sampling and exploration of NWMs to evaluate the presence of CMVs and to confirm the usefulness of those sequences as a new molecular tool to infer the systematics of Platyrrhini.

MATERIALS AND METHODS

Sample collection. The collection of blood DNA samples has been described in detail elsewhere (**Table 1**) (35, 36, 43-45). In brief, we tested a total of 244 DNA samples from 20 NWM species (26 subspecies) belonging to the three families and to six of the seven subfamilies according to Schneider and Sampaio (5). All samples were previously genetically identified by phylogenetic analysis of mtDNA genes, including cytochrome *c* oxydase subunit I (*COX1*) and/or cytochrome *b* (*CytB*) (35).

Initial screening of samples. Molecular screening was done by semi-nested polymerase chain reaction amplification with degenerate consensus primers targeting highly conserved amino acid (aa) motifs of the herpesvirus *DNA polymerase* gene (**Table 2**). To maximize the chances of amplifying CMV-like sequences, the primers of Rose *et al.* were refined from the alignment of all primate cytomegalovirus DNA polymerase sequences available in the databases (**Table 2**) (46). CMV3F1, CMV3F2 and CMV3R1 primers were designed and used in place of QAHNA, VYGA and GDTD1B, respectively, while the sense primer DFASA was kept as it was. Two different combinations of primers (DFASA/ CMV3R1 or CMV3F1/ CMV3R1) were used on each DNA sample in separate reactions for the first-round PCR (**Figure 2**). In the second-round PCR, CMV3F2/CMV3R1 primers were used. PCR analyses were performed at an annealing temperature of 60°C, with an elongation time of 30 s, for 35 cycles. All amplicons of approximately the expected size were purified, cloned by TA cloning and sent for sequencing to Genewiz®, Takeley, UK (https://www.genewiz.com/).

Partial *DNA polymerase* gene amplification. To obtain the nucleotide sequence upstream of the CMV3F2 motif, a degenerate primer (CMV3R2) was derived from the complementary sequences of the small fragments and used in an nPCR amplification with the DFASA or CMV3F1 primer pools using the initial PCR products as templates (**Table 2, Figure 2**). Then, to generate longer segments of the *DNA polymerase* gene for each newly identified virus, we tried to obtain upstream and downstream sequences using different sets of consensus degenerate and species-specific primers designed using the primate CMV DNA polymerase sequence alignment (**Table 2, Figure 2**). Overlapping amplicons were generated, cloned and sequenced as described above. Each sequence corresponds to at least three independent clones sequenced on both strands. Contig sequences were then assembled using MEGA 5.05 software (47).

Phylogenetic analysis. Raw sequences were analyzed and edited in MEGA 5.05 (47). Sequences were confirmed as CMV by homology analysis using the NCBI BLAST search tool (48). Then multiple sequence alignments were constructed using ClustalW with all other previously published primate CMV sequences and alignments were checked manually. Sequences were translated into amino acids and both nucleotide and amino acid sequences were checked for irregularities. Hypervariable regions were removed before performing analyses. Sequence identity was calculated using uncorrected p-distances. Phylogenetic trees were inferred from the aligned amino acid sequences. The JTT + G model was selected as the best-fitted model of amino acid evolution under corrected Akaike information criteria (AICc) using MEGA 5.05 and used for the Bayesian approach (49), which was performed with Mr. Bayes 3.2.2 to infer phylogenetic relationships (50). Markov Chain Monte Carlo (MCMC) simulations were run for 10,000,000 generations, with four simultaneous chains, using a sample frequency of 500 and a burn-in of 25,000. Majority rule consensus trees were obtained from the output. Validation of the

inference was assessed based on the standard deviation of split frequencies, which was less than the expected threshold value of 0.01 (calculated value of 0.002).

Time calibration. Divergence times between clades were calculated using a relaxed Bayesian molecular clock model with an uncorrelated lognormal rate of distribution, as implemented in BEAST version 1.7.4 (51). A monophyletic constraint was imposed for the nodes used to calibrate evolutionary rates. Two calibration points were applied as normal prior to constraining the age of the Platyrrhini and Homo-Pan clades, the time of the most recent common ancestor (tMRCA) of Platyrrhini to 23.5 MYA (stdev = 3.0) and for Homo-Pan to 6.5 MYA (stdev = 0.8) (52-54). These calibration points are based on the fossil date (55). The amino acid substitution model was the same as the one described above. A Yule process of speciation was used as the tree prior. Results were obtained for 10,000,000 generations with the first 2,500,000 discarded as burn-in and parameter values sampled every 100 generations. The effective sample size for parameter estimates and convergence were checked using Tracer version 1.5.0 software (56). The final tree with divergence estimates and their 95% highest posterior densities was computed in TreeAnnotator v1.4.5 (51).

Accession numbers. The sequences reported in this paper have been deposited in the GenBank database under accession numbers **KU963225** to **KU963240**.

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COMPETING INTERESTS

The authors declare that they have no competing financial interests.

AUTHORS’ CONTRIBUTION

A.L., M.R-G. and V.L. contributed to the study design. M.R-G. collected some of the samples. S.J., D.D., J.-F.P., A.L. and V.L. performed the molecular and genetic analyses. S.J., A.L. and V.L. analyzed the data and wrote the article. All authors participated in its final writing and editing.

ETHICAL APPROVAL

Study based on samples collected several years earlier. Biological material from French Guiana was collected in 1994–1995 along the Sinnamary River, Petit Saut Hydroelectric dam, under the supervision of veterinarians of “Faune Sauvage” team led by Jean-Christophe Vié (57). Blood

sampling from live animals was carried out in accordance with French animal care regulations and the laws of France. The other samples were collected directly from animals killed, in the field, by indigenous hunters for their own purposes, with the full consent of the hunters and in accordance with the laws of Brazil, Colombia, Mexico, Peru, Guatemala and Argentina.

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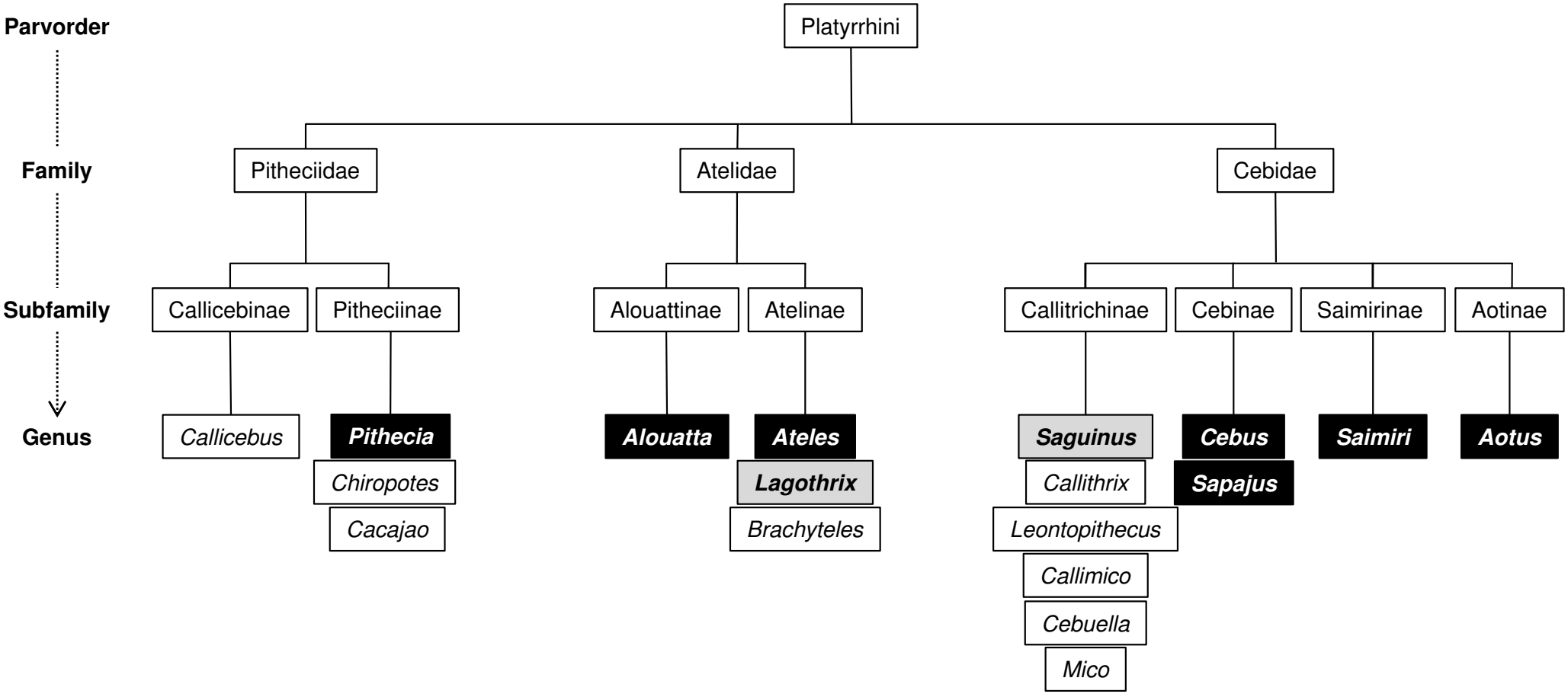
FIGURE LEGEND

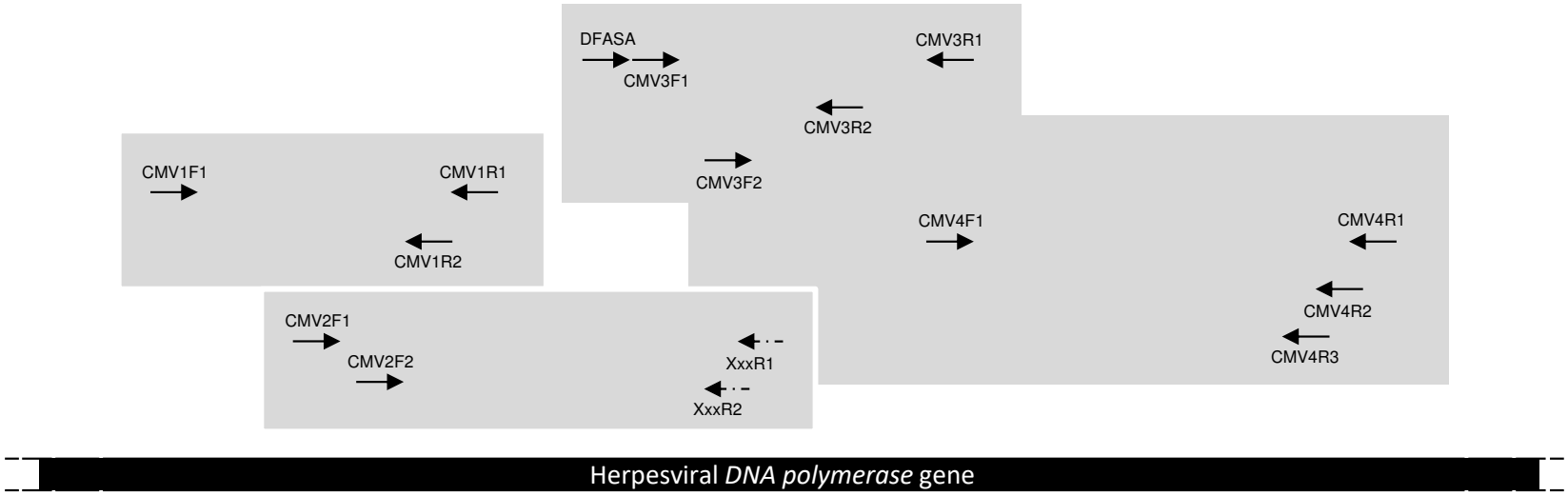
FIG.1. Diagram representation of Plathyrrhini taxa in descending order down to genus level. Adapted from Perelman et al. (3). Black and gray boxes represent NWM genera tested for CMVs. Black boxes correspond to NWM genera from which CMV sequences have been characterized while gray boxes represent NWM genera from which no CMV sequence has been obtained in the present study.

FIG. 2. Relative position and orientation of the PCR primers used. The different combinations of primers used in nested or semi-nested PCRs are represented above the Herpesviral *DNA polymerase* gene sequence in different grey boxes. Primers XxxR1 and XxxR2, represented by dotted arrows, correspond to the different antisense specific primers used in a degenerate (CMV2F1 or CMV2F2) - nondegenerate nPCR assay (see **Table 2**). Bars below the sequence represent the different nested PCR products expected. The pairs of primers on the left-hand side of the bars indicate those used for the first-round PCR, while those on the right-hand side correspond to the one used in the nested PCR reactions. The sequences of the oligonucleotide primers are given in **Table 2**.

FIG. 3. Phylogenetic analysis of primate cytomegalovirus sequences. The phylogenetic tree was derived from the partial amino acid sequences of the *DNA polymerase* gene (149 aa) of 50 representatives of primate cytomegaloviruses using the Bayesian method with the JTT + G model of amino acid evolution. Sequences generated in this study are in boldface. Posterior probabilities of the Bayesian analysis (>0.9) are shown next to each node. The scale bar indicates amino acid substitutions per site. The major clades representing Old World and New World primate families,

631 super-families (for OWM) and parvorders are labeled on the right-hand side of the figure. The
632 virus names are associated with their accession numbers. Viruses of Cercopithecidae comprise
633 those of the chacma (BaCMV NC_027016 (20, 58)) and olive (BaCMV AF387664 (21))
634 baboons, the moustached guenon (CceCMV1 AY728178), the agile mangabey (CagCMV1
635 AY608713), the drill (MndCMV AF282941 and MndCMV AF387665 strain OCOM6-2 (21,
636 27)), the mandrill (MndCMV AY129399), the African green monkey (CeHV5 AY117754,
637 CeHV5 FJ483969 strain Colburn, CeHV5 FJ483969 strain 2715, CaeCMV AF292066,
638 VervetCMV AY049066 strain CSG and CeHV3 AY049065 (21, 59)), the cynomolgus
639 (MfasCMV1 JN227533 strain Ottawa, MfasCMV1 AY728171 and MfasCMV KP796148 strain
640 Mauritius (60, 61)) and rhesus (McHV3 AF033184 and McHV3 DQ120516 isolate CMV 180.92
641 (62, 63)) macaques and the mantled guereza (CgueCMV1.1 AY129397 and CgueCMV1.2
642 EU118147 (29)). Viruses of Hominidae comprise those of the Bornean orangutan (PpygCMV1.1
643 AY129396), the human (HHV5 M14709 strain AD169, HHV5 NC_006273 strain Merlin, HHV5
644 AY315197 strain Towne and HHV5 AC146905 isolate Toledo (64-67)), the Western gorilla
645 (GgorCMV2.1 FJ538490 (26)) and the common chimpanzee (PnHV2 AF480884 strain
646 Heberling, PtroCMV1.1 FJ538485 and PtroCMV AF292063 (26, 65)). Regarding viruses of New
647 World monkeys, in addition to the ones describe in the present study, viruses of Cebidae
648 comprise those of the capuchin monkey (CebHV1 JQ264772 and CebusHV AF292067 from
649 *Cebus* sp.), the common squirrel monkey (SaHV4 FJ483967 and SscHV AF292065) and the
650 three-striped night monkey (AoHV1 FJ483970).


Parvorder
Family
Subfamily
Genus
Platyrrhini
Pitheciidae
Atelidae
Cebidae
Callicebinae
Pitheciinae
Alouattinae
Atelinae
Callitrichinae
Cebinae
Saimirinae
Aotinae
Callicebus
Pithecia
Chiropotes
Cacajao
Alouatta
Ateles
Lagothrix
Brachyteles
Saguinus
Callithrix
Leontopithecus
Callimico
Cebuella
Mico
Cebus
Sapajus
Saimiri
Aotus



DFASA/CMV3R1 or CMV3F1/CMV3R1 — CMV3F2/CMV3R1

DFASA/CMV3R1 — DFASA/CMV3R2

CMV3F1/CMV3R1 — CMV3F1/CMV3R2

CMV1F1/CMV1R1 — CMV1F1/CMV1R2

CMV2F1/XxxR1 — CMV2F2/XxxR2

CMV3F2/CMV4R1 — CMV3F2/CMV4R2

CMV3F2/CMV4R1 — CMV3F2/CMV4R3

CMV3F2/CMV4R1 — CMV4F1/CMV4R1

CMV3F2/CMV4R1 or CMV4F1/CMV4R1 — CMV4F1/CMV4R2

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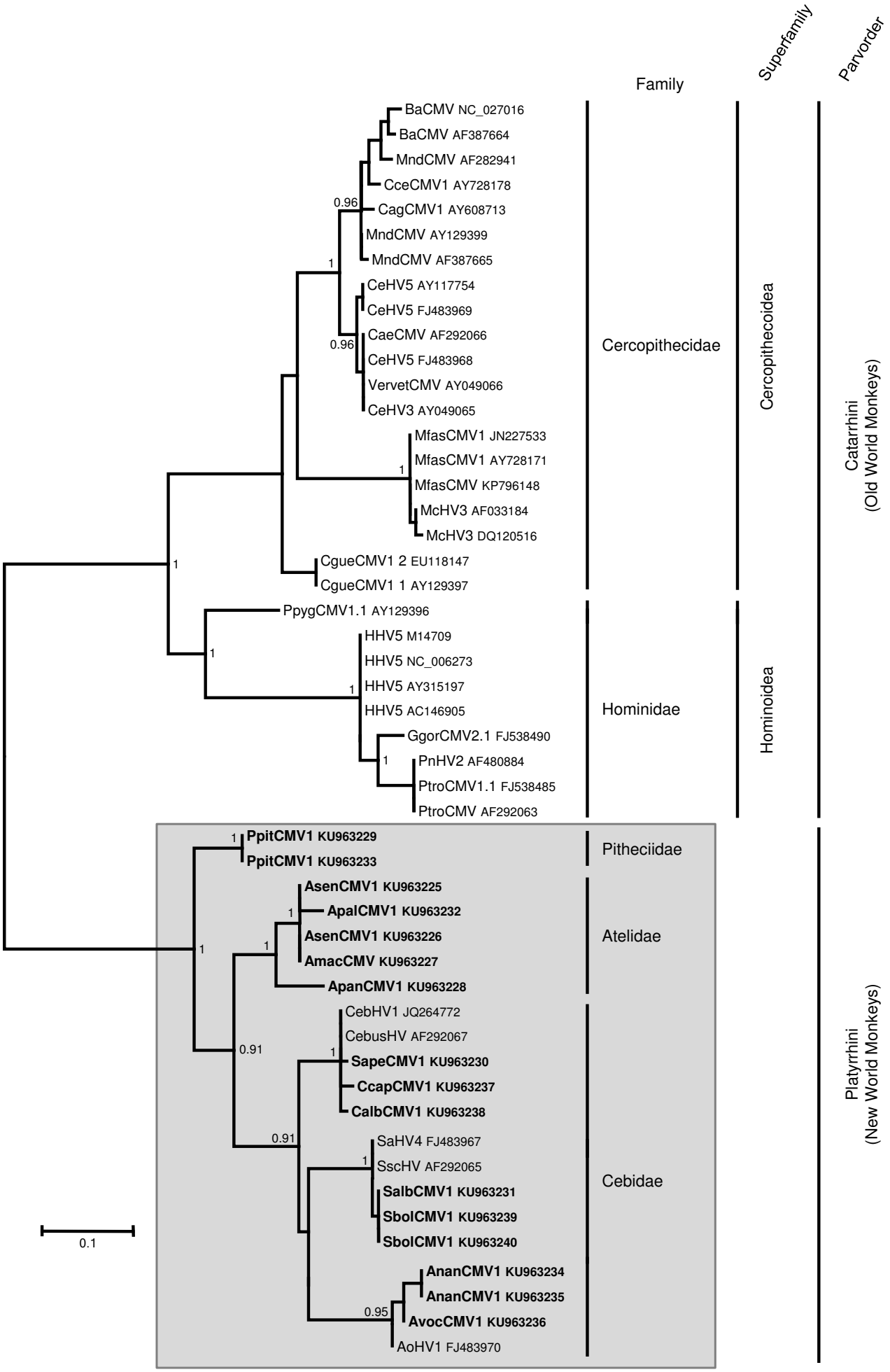

Family
Superfamily
Parvorder
BaCMV NC_027016
BaCMV AF387664
MndCMV AF282941
CceCMV1 AY728178
CagCMV1 AY608713
MndCMV AY129399
MndCMV AF387665
CeHV5 AY117754
CeHV5 FJ483969
CaeCMV AF292066
CeHV5 FJ483968
VervetCMV AY049066
CeHV3 AY049065
MfasCMV1 JN227533
MfasCMV1 AY728171
MfasCMV KP796148
McHV3 AF033184
McHV3 DQ120516
CgueCMV1 2 EU118147
CgueCMV1 1 AY129397
PpygCMV1.1 AY129396
HHV5 M14709
HHV5 NC_006273
HHV5 AY315197
HHV5 AC146905
GgorCMV2.1 FJ538490
PnHV2 AF480884
PtroCMV1.1 FJ538485
PtroCMV AF292063
PpitCMV1 KU963229
PpitCMV1 KU963233
AsenCMV1 KU963225
ApalCMV1 KU963232
AsenCMV1 KU963226
AmacCMV KU963227
ApanCMV1 KU963228
CebHV1 JQ264772
CebusHV AF292067
SapeCMV1 KU963230
CcapCMV1 KU963237
CalbCMV1 KU963238
SaHV4 FJ483967
SscHV AF292065
SalbCMV1 KU963231
SbolCMV1 KU963239
SbolCMV1 KU963240
AnanCMV1 KU963234
AnanCMV1 KU963235
AvocCMV1 KU963236
AoHV1 FJ483970
Cercopithecidae
Hominidae
Pitheciidae
Atelidae
Cebidae
Cercopithecoidea
Hominoidea
Catarrhini
(Old World Monkeys)
Platyrrhini
(New World Monkeys)
0.96
0.96
1
1
1
1
1
1
1
1
1
1
0.91
0.91
0.95
0.1

TABLE 1. New World non-human primates tested for cytomegaloviruses using molecular methods and survey results

Primate Taxonomy						Common Name	Origin	Number[b]	Size[c]	Informal names		Accession numbers
O sO iO pO	Family[a]	Subfamily	Genus	species	subspecies					Name	Acronym	
Primates Haplorrhini Simiiformes Platyrrhini	Cebidae	Callitrichinae	*Saguinus*	*midas*		Red-handed tamarin	French Guiana	0/54				
				labiatus		White-lipped tamarin	Peru	0/2				
				oedipus		Cottontop tamarin	Colombia	0/2				
		Cebinae	*Sapajus*[d]	*apella*		Tufted capuchin	French Guiana	0/5				
				apella		Tufted capuchin	Colombia	1/10	910	*S. apella* CMV1	**SapeCMV1**	KU963230
			Cebus	*albifrons*		White-fronted capuchin	Colombia	1/10	448	*C. albifrons* CMV1	**CalbCMV1**	KU963238
				capucinus		White-headed capuchin	Colombia	1/10	448	*C. capucinus* CMV1	**CcapCMV1**	KU963237
		Saimirinae	*Saimiri*	*boliviensis*	*boliviensis*	Black-capped squirrel monkey	Colombia	2/3	448	*S. b. boliviensis* CMV1	**SbolCMV1**	KU963239, KU963240
				sciureus	*sciureus*	Common squirrel monkey	French Guiana	0/4				
				sciureus	*albigena*	Colombian common squirrel monkey	Colombia	1/1	910	*S. s. albigena* CMV1	**SalbCMV1**	KU963231
				sciureus	*macrodon*	Ecuadorian common squirrel monkey	Colombia	0/1				
		Aotinae	*Aotus*	*vociferans*		Spix's night monkey	Peru	1/5	451	*A. vociferans* CMV1	**AvocCMV1**	KU963236
				nancymaae		Nancy Ma's night monkey	Peru	2/6	964	*A. nancymaae* CMV1	**AnanCMV1**	KU963234, KU963235
	Pitheciidae	Pitheciinae	*Pithecia*	*pithecia*		White-faced saki	French Guiana	2/4	916	*P. pithecia* CMV1	**PpitCMV1**	KU963229, KU963233
	Atelidae	Alouattinae	*Alouatta*	*macconnelli*		Guyanan red howler	French Guiana	5/94	1724	*A. macconnelli* CMV1	**AmacCMV1**	KU963227
				seniculus		Venezuelan red howler	Colombia	2/8	1724	*A. seniculus* CMV1	**AsenCMV1**	KU963225, KU963226
				caraya		Black howler	Argentina	0/1				
				palliata		Mantled howler	Mexico	1/2	988	*A. palliata* CMV1	**ApalCMV1**	KU963232
		Atelinae	*Ateles*	*paniscus*		Red-faced spider monkey	French Guiana	1/5	2026	*A. paniscus* CMV1	**ApanCMV1**	KU963228
				belzebuth		White-fronted spider monkey	Colombia	0/3				
				fusciceps	*robustus*	Black-headed spider monkey	Colombia	0/4				
				geoffroyi		Geoffroy's spider monkey	Guatemala	0/3				
			Lagothrix	*lagotricha*	*cana*	Gray woolly monkey	Brazil	0/1				
				lagotricha	*lagotricha*	Brown woolly monkey	Colombia	0/2				
				lagotricha	*lugens*	Colombian woolly monkey	Colombia	0/2				
				lagotricha	*poeppigii*	Silvery woolly monkey	Peru	0/2				

Abbreviations: O, order; sO, suborder; iO, infraorder; pO, parvorder; CMV, Cytomegalovirus.

[a] According to Perelman *et al.* (3).

[b] Number: number of CMV-positive animals (by PCR, cloning and sequencing)/number of tested animals.

[c] Size: size of the *DNA polymerase* gene fragments obtained, in bp.

[d] According to Alfaro *et al.* (16).

TABLE 2. Oligonucletide primers used for Cytomegalovirus *DNA polymerase* consensus and specific PCRs

Oligonucleotide	Orientation[a]	Location[b]	5' -> 3' sequence[c]	CMV sequence amplified
DNA pol degenerate primers				
CMV1F1	+	721 - 749	GAC AAG AAG TTG ACN ACN TTY GGN TGG TG	AmacCMV1,AsenCMV1, ApanCMV1
CMV1R1	-	1534 - 1559	ACG CCG GCY TCR TAR TGR AAR TTD AT	AmacCMV1,AsenCMV1, ApanCMV1
CMV1R2	-	1480 - 1504	CGT CCT GHA CRC ART AYT TNC CNA C	AmacCMV1,AsenCMV1, ApanCMV1
CMV2F1	+	1330 - 1352	GAY ATG TAY CCN GTS TGY ATG GC	AmacCMV1, AsenCMV1, ApanCMV1, PpitCMV1, SapeCMV1, SalbCMV1
CMV2F2	+	1372 - 1394	TAC AAR YTV AAY ACB ATG GCS GA	AmacCMV1, AsenCMV1, ApanCMV1, PpitCMV1, SapeCMV1, SalbCMV1
DFASA[d]	+	1768 - 1793	GTG TTC GAC TTY GCN AGY YTN TAY CC	All
CMV3F1	+	1795 - 1817	TCH ATY ATY ATG GCN CAY AAY CT	All
CMV3F2	+	2062 - 2090	ACG TGC AAT TCT TTY TAY GGB TTY ACN GG	All
CMV3R1	-	2269 - 2303	CGA TAG CAC ACA AAC ACR CTR TCN GTR TCN CCR TA	All
CMV3R2	-	2125 - 2147	CCG ATD CGN GTR ATR CTR GCC GC	All
CMV4F1	+	2266 - 2291	ATC TAY GGK GAC ACS GAY AGY GTS TT	AmacCMV1, AsenCMV1, ApalCMV1, AnanCMV1, ApanCMV1
CMV4R1	-	2782 - 2801	GCC GCY ARN CGY TTD ATG AC	ApalCMV1, AnanCMV1, ApanCMV1
CMV4R2	-	2477 - 2498	CGC ACC ARR TCR ACN CCY TTC A	AmacCMV1, AsenCMV1, ApalCMV1, AnanCMV1, ApanCMV1
CMV4R3	-	2419 - 2444	ATA TAC CGY TTY TTR CAG ATC ATC AT	AmacCMV1, AsenCMV1, ApalCMV1, AnanCMV1, ApanCMV1
DNA pol antisense specific primers (in combination with CMV2F1 or CMV2F2) [e]				
PitR1	-	1958 - 1978	TGC GCT GAG CAA CCC ATT TAG	PpitCMV1
PitR2	-	1912 - 1932	ACG CAC CTC CGA CTT CAC AAA	PpitCMV1
AotR1	-	2019 - 2039	TTG TCG AGC AGC GTC CTC TTG	AnanCMV1, AvocCMV1
AotR2	-	1884 - 1904	ACC GTA CCG TTT TCG AAG TTA	AnanCMV1, AvocCMV1
SapR1	-	2106 - 2126	GCG ACT GGC AAA CAC GGT AAC	SapeCMV1
CapR2	-	1994 - 2016	GGG ATC TGT GCA ATC TTT CAT GG	CalbCMV1, CcapCMV1
CapR3	-	1939 - 1961	CGG GTC AAC AAT TCA GAA AGC AC	SapeCMV1, CalbCMV1, CcapCMV1
AteR1	-	1994 - 2016	CGG ATC TCT GCA ATT TTT CAT GG	ApanCMV1
AteR2	-	1935 - 1957	TCA GCA GTT CGG ACA ACA CTG AA	ApanCMV1
SaiR1	-	1948 - 1969	CCA CCC ACT TCG TTA GCA GCT C	SbolCMV1, SalbCMV1
SaiR2	-	2155 - 2175	ACG CGC GGT GTC TTG TAA CAT	SbolCMV1, SalbCMV1
AloR1	-	1959 - 1979	TTC CGT TGA GCC ACC CAT TTA	AmacCMV1, AsenCMV1, ApalCMV1
AloR2	-	1932 - 1954	GCA ATT CCG ATA GCA CTG AGG AA	AmacCMV1, AsenCMV1, ApalCMV1

[a] +, sense; -, antisense.

[b] Positions relative to ATG of the *DNA polymerase* gene of AoHV1, accession number FJ483970.

[c] Positions of degeneracy follow the International Unit Base codes.

[d] Degenerate oligonucleotide primer described in Rose *et al.* (45).

[e] For clarity, all antisense specific primers are indicated as XxxR1 for R1 primers and XxxR2 for R2 primers in Figure 2.

TABLE 3. Nucleotide and amino acid identities between the novel Cytomegaloviruses and all other NHP Cytomegaloviruses and HCMV[a]

			% identity[b] with													
		Viruses of	***Sapajus*** (SapeCMV1)		***Cebus*** (CalbCMV1, CcapCMV1)		***Saimiri*** (SbolCMV1, SalbCMV1)		***Aotus*** (AvocCMV1, AnanCMV1)		***Pithecia*** (PpitCMV1)		***Alouatta*** (AmacCMV1, AsenCMV1, ApalCMV1)		***Ateles*** (ApanCMV1)	
			Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid
Cd[c]	Cn	*Sapajus*			87.6 - 88.7	98.0 - 99.3	76.1 - 77.0	89.1	74.3 - 75.4	85.7	72.9 - 73.1	83.7	74.5 - 74.7	83.0 - 85.0	75.9	80.3
		Cebus[d]	87.6 - 88.7	98.0 - 99.3	95.7 - 99.6	98.0 - 99.3	72.2 - 77.2	87.1 - 88.4	74.0 - 75.4	85.0 - 87.1	72.0 - 72.5	83.0 - 84.4	74.3 - 75.4	81.6 - 83.7	74.5 - 74.7	79.6 - 81.0
	Sn	*Saimiri*[e]	76.1 - 77.4	89.1	75.2 - 77.7	87.1 - 87.8	97.1 - 97.7	99.3 - 100	74.3 - 74.9	85.71	74.7 - 75.6	82.31	74.7 - 76.1	85.0 - 85.7	75.2 - 75.6	83.67
	An	*Aotus*[f]	74.3 - 75.4	85.7 - 86.4	74.0 - 75.6	85.0 - 87.8	74.0 - 74.7	85.7 - 86.4	94.8 - 96.2	96.6 - 98.6	74.0 - 74.7	83.7 - 85.0	71.8 - 74.8	79.6 - 82.3	71.1 - 72.2	81.63
Pd	Pn	*Pithecia*	72.9 - 73.1	83.7	72.0 - 72.5	83.0 - 83.7	74.72	82.3 - 83.3	74.0 - 74.7	84.4 - 85.0	99.6	100	72.9 - 75.2	83.0 - 85.0	75.2 - 75.4	84.35
Ad	Al	*Alouatta*	74.5 - 74.7	82.3 - 83.0	74.3 - 75.4	81.6 - 83.0	74.9 - 76.1	85.0 - 85.7	71.8 - 73.1	79.6 - 81.6	72.9 - 75.2	83.0 - 85.0	92.1 - 98.9	97.3 - 100	82.2 - 83.5	91.2 - 92.5
	At	*Ateles*	75.9	80.3	74.5 - 74.7	79.6 - 80.3	75.4 - 75.6	83.67	71.1 - 71.6	81.63	75.2 - 75.4	84.35	82.2 - 83.5	91.2 - 92.5	-	-
Hd[g]	Hn	*Homo*	64.1 - 64.6	67.35	64.3 - 65.9	66.7 - 68.0	63.2 - 64.3	67.35	70.0 - 70.9	72.1 - 72.8	65.2 - 66.1	70.07	61.6 - 63.7	65.99	62.1 - 63.0	67.35
		Pan	61.85	66.67	63.2 - 63.7	66.0 - 66.7	63.2 - 63.4	66.67	71.6 - 72.0	71.4 -72.1	66.1 - 66.6	70.07	61.2 - 62.7	66.67	62.08	68.03
		Gorilla	62.98	67.35	63.7 - 63.9	66.7 - 68.0	62.3 - 62.5	67.35	71.33	72.1 - 72.8	62.98	70.75	61.0 - 62.1	65.99	60.95	68.03
	Po	*Pongo*	64.11	69.39	63.4 - 64.1	69.4	65.5 - 65.7	68.03	70.4 -70.7	71.43	66.14	69.39	62.5 - 63.4	68.0 - 68.7	62.53	68.71
Ce[g]	Cr	*Macaca*	62.1 - 63.4	63.3 - 64.0	59.8 - 61.6	61.9 - 63.3	62.1 - 63.2	64.6 - 65.3	65.9 - 66.6	65.3 - 67.4	63.4 - 63.9	64.6 - 65.3	60.3 - 62.1	64.6 - 66.7	62.5 - 63.0	64.6 - 65.3
		Papio	64.8 - 66.4	64.0 - 64.6	62.1 - 63.4	61.9 - 63.3	62.1 - 62.3	66.0 - 66.7	68.2 - 69.1	64.0 - 65.3	64.6 - 64.8	65.3 - 66.0	62.3 - 63.4	66.0 - 66.7	62.53	65.3
		Mandrillus	64.1 - 65.7	64.0 - 66.0	61.8 - 63.4	61.9 -64.6	61.4 - 63.0	66.7 - 68.0	67.5 - 68.8	64.6 - 67.4	64.3 - 65.9	66.0 - 68.0	60.9 - 64.6	66.0 - 68.0	61.8 - 62.7	65.3 - 67.4
		Cerco/Chloro	63.7- 65.5	64.6 - 66.0	61.6 - 68.8	63.3 - 65.3	60.9 - 63.7	66.7 - 68.0	67.5 - 69.3	65.3 - 68.0	63.7 - 65.5	66.7 - 68.7	61.8 - 65.0	66.7 - 68.7	60.5 - 63.2	66.0 - 68.0
	Co	*Colobus*	63.9 - 64.1	65.3	63.9	63.3 - 65.3	63.7 - 64.6	68.7	72.2 - 72.7	68,0	66.1 - 66.4	66.7	63.4 - 66.1	67.4 - 68.0	62.5 - 62.8	66.7

[a] Numbers refer to values obtained in comparison with the 447-bp fragment of the conserved *DNA polymerase* gene that is available for all viruses.

[b] Sequences identified from specimens from the same primate species showing 100% nucleotide identity *e.g.* SbolCMV1, AnanCMV1, AmacCMV1 and AsenCMV1 are not included.

[c] Abbrevations, Cd, Cebidae; Cn, Cebinae; Sn, Saimiriinae; An: Aotinae; Pd, Pitheciidae; Pn, Pitheciinae; Ad, Atelidae; Al, Alouattinae; At, Atelinae; Hd, Hominidae; Hn, Homininae; Po, Pongidae; Ce, Cercopithecidae; Cr; Cercopithecinae; Co, Colobinae.

[d] Nucleotide and amino acid identities of viruses of Cebus rely on the sequences generated in this study as well as on sequences of CebHV1 $_{JQ264772}$ and CebusHV $_{AF292067}$.

[e] Nucleotide and amino acid identities of viruses of Saimiri rely on the sequences generated in this study as well as on sequences of SaHV4 $_{FJ483967}$ and SscHV $_{AF292065}$.

[f] Nucleotide and amino acid identities of viruses of Aotus rely on the sequences generated in this study as well as on the sequence of AoHV1 $_{FJ483970}$.

[g] Hominidae and Cercopithecidae viral sequences used to calculate nucleotide and amino acid identities correspond to those shown in Figure 3. Their GenBank accession numbers and associated publications are all reported in the figure and its legend.

TABLE 4. Estimates of Platyrrhini divergence time from CMV *DNA polymerase* sequence data and comparison with other estimates

Node	This study	Perelman et al. (3)	Jameson Kiesling et al. (15)
Catarrhini / Platyrrhini	32.45[a] (17.76 - 52.33)[b]	43.47 (38.55 - 48.36)	37.72 (36.04 - 42.07)
Pitheciidae / Atelidae+Cebidae	22.33 (16.25 - 28.21)	24.82 (20.55 - 29.25)	25.51 (25.14 - 26.36)
Atelidae / Cebidae	17.16 (10.29 - 24.25)	22.76 (18.14 - 27.08)	24.04 (22.6 - 25.29)
Atelinae / Alouattinae	8.29 (2.57 - 14.82)	16.13 (10.52 - 21.35)	15.29 (13.29 - 17.99)
Within *Alouatta*	3.11 (0.47 - 6.82)	ND	5.14 (3.65 - 6.8)
Within Cebidae	11.54 (5.82 - 17.89)	19.95 (15.66 - 24.03)	20.86 (18.48 - 22.86)
Within *Aotus*	3.98 (0.8 - 8.25)	5.54 (3.20 - 7.85)	4.39 (3.12 - 5.75)
Within *Cebus*	3.26 (0.59 - 6.94)	6.00 (3.13 - 9.35)	5.19 (3.69 - 6.78)
Within *Saimiri*	2.68 (0.28 - 6.14)	2.24 (1.05 - 3.73)	0.97 (0.51 - 1.45)

[a] times in units of millions of years

[b] values given in the parentheses indicate 95% HPD in millions of years.