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Animal enteroviruses: a glimpse of a broad evolutionary landscape

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Abstract

The genus *Enterovirus* (family *Picornaviridae*) contains numerous viruses, most of which have been identified in humans. Among them, the three serotypes of poliovirus, coxsackieviruses A and B, echoviruses, rhinoviruses and other enteroviruses (EVs) responsible in humans for a wide spectrum of clinical manifestations. There are also 60 identified EVs in different mammals. Some have been found in both humans and animals, demonstrating the possibility of zoonotic transmission of certain EVs.

Compared to human EVs, genetic and epidemiological data for animal EVs are scarce. However, the detection of EVs in various species of mammals and their presence on all continents suggest that the number of EVs still to be discovered is very important. Some EVs found in animals have characteristics never seen in human EVs. Furthermore, the unique phylogenetic relationships observed between animal EVs raise interesting questions about the rules that govern the evolution of these viruses. The aim of this review is to present the salient data on animal EVs and to highlight the questions they raise.

Keywords: enterovirus, coxsackievirus, recombination, *Picornaviridae*, Swine Vesicular Disease Virus, zoonosis

Entérovirus des animaux : lumière sur l'immensité de l'espace évolutif des entérovirus

Résumé

Le genre *Enterovirus* famille *Picornaviridae*) regroupe de nombreux virus dont la plupart ont été identifiés chez l'Homme. Parmi eux, les trois sérotypes de poliovirus, les coxsackievirus A et B, les echovirus, les rhinovirus et d'autres entérovirus (EV) sont responsables chez l'Homme d'un large spectre d'affections plus ou moins sévères. Il existe également une soixantaine d'EV identifiés chez différentes espèces de mammifères. Certains EV ont été trouvés à la fois chez l'Homme et l'animal, démontrant la possibilité d'une transmission zoonotique.

Les données génétiques et épidémiologiques relatives aux EV animaux sont rares. La détection d'EV dans diverses espèces de mammifères sur tous les continents suggère que la diversité de ces virus est sous-estimée. Certains EV trouvés chez les animaux présentent des caractéristiques spécifiques jamais observées chez les EV humains. Par ailleurs, les relations phylogénétiques singulières observés entre des EV animaux soulèvent d'intéressantes questions concernant l'histoire de ces virus. L'objectif de cette revue est de synthétiser les données saillantes concernant les EV circulant chez les animaux et les principales questions que soulèvent leurs spécificités génomiques, virologiques et phylogénétiques.

Mots clés : entérovirus, coxsackievirus, recombinaison, *Picornaviridae*, Swine Vesicular Disease Virus, zoonose

The genus *Enterovirus* (family *Picornaviridae*) contains many viruses infecting mammals. The first enteroviruses (EVs) identified were polioviruses, the causative agents of poliomyelitis, which were cultivated in laboratories as soon as 1948. The search for polioviruses during poliomyelitis epidemics in the 1950s led to the identification of many other EVs infecting humans. At the same time, the first animal EVs were discovered in the faeces of farm animals and in non-human primates (NHPs) used in laboratories. Since then, many EVs have been found in many mammalian species. However, most of the known EVs have been identified in domestic animals or in animals living in the vicinity of humans, suggesting that much greater diversity exists, particularly in wildlife. Although genetic data about animal EVs are relatively scarce compared to those available for human EVs, their analysis reveals original traits in some animal EVs. The purpose of this review is to provide an overview of the known animal EVs and to highlight their major characteristics and the scientific questions they raise.

EV features

The viral particles of EVs are icosahedral and non-enveloped. They contain a copy of the genome, which consists of a single-stranded RNA molecule of 7,000-8,000 nucleotides. The viral genome contains a long open reading frame that encodes a polyprotein containing the four structural proteins that form the capsid (VP1 to VP4) and all the non-structural proteins involved in the viral cycle (Figure 1). An additional short open reading frame has been detected in some EVs; it encodes a protein not essential to the viral cycle [1].

The 5' extremity of the EV genome has no cap but is covalently bound to a viral protein, VPg. The 5' untranslated region (5'UTR) of the genome folds to form three-dimensional structures essential for genome replication and translation (Figure 1): at the very beginning of the genome, a cloverleaf is essential for replication; downstream, a series of

structures form the internal ribosome entry site (IRES), which allows cap-independent translation initiation [2].

Concept of species and virus types within the genus *Enterovirus*

The current classification of EVs is based on their phylogenetic relationships and is organized by the International Committee for the Taxonomy of Viruses (ICTV). Result of a long history punctuated by several modifications [3], the current taxonomic classification assigns EV strains into 15 viral species including rhinoviruses (EV-A to EV-L and RV-A to RV-C) [4]. The criteria currently used to define a species within the genus *Enterovirus* are indicated in the boxed text [5]. The classification mainly relies on the capsid-encoding genomic region for two reasons: 1- recombinant events being particularly common between EVs, the different genomic regions (5'UTR, capsid-encoding region, regions encoding non-structural proteins) follow different evolutionary pathways [6]; 2- the viral particles being non-enveloped, it is the capsid that confers its main characteristics to the virion (cell receptors, thermosensitivity, sensitivity to acidic pH, neutralizing epitopes, etc.).

Historically, serotypes were differentiated within each species, based on antigenic characteristics established by *in vitro* seroneutralization tests. In the late 1990s, it was shown that the serological classification was broadly consistent with the phylogenetic classification based on the capsid-encoding nucleotide sequences, especially the VP1-encoding region. Since then, molecular typing has supplanted serotyping and EVs are now classified into “virus types” rather than in “serotypes”: two viruses belong to the same virus type if they are relatively close one with the other at the nucleotide and peptide levels in the capsid [7,8]. To date, more than 300 distinct types have been identified within the genus *Enterovirus*.

Species EV-E et EV-F

The species EV-E and EV-F were created during a drastic overhaul of the genus *Enterovirus* in 2017 (10th ICTV report). These two species are comprised of virus types originally combined into a single species called *Bovine enterovirus* [9].

EV-Es are known through a hundred of specimens. Most of them come from Asia (China, Thailand, Pakistan, Japan, Turkey) but EV-Es have also been detected in the United States, Brazil, Europe, Australia and New Zealand. The majority of EV-Es have been detected in cattle (*Bos taurus*), but one EV-E specimen was detected in the faeces of a rhesus macaque in Bangladesh [10] and another one in a dolphin (*Tursiops truncatus*) living in a coastal open ocean water enclosure in the United States [11]. The low genetic distance between these two EV-E strains and EV-Es circulating in cattle does not support the existence of EV-E populations circulating specifically in primates or marine mammals. Rather, it would be sporadic interspecies transmissions. Several studies have shown anti-EV-E antibodies in donkeys, horses, goats, sheep, camelids, dogs and even in humans [12–14]. Although these studies show higher rates of seropositivity in cattle than in other animals living in the same regions [13–15], they suggest the ability of EV-Es to infect a wide variety of mammal hosts, including primates. However, the lack of data on possible serological cross-reactivity between EV-Es and EVs of other species calls for careful consideration of serology-based results in the absence of molecular detection or virus isolation.

The number of EV-F specimens is limited to a few dozen from the United States, Europe, Egypt, Nigeria, China, Japan, Bangladesh and New Zealand. Most EV-Fs have been detected in *B. taurus* but some have also been observed in yaks [16], an alpaca [17], a goat [18], a monkey (*Trachypithecus pileatus*) [10] and a marsupial (*Trichosurus vulpecula*) [19].

Genetic sequences close to those of EV-Es and -Fs were detected in faeces of geese, deer, sheep, horse and Indian bison; however, the region sequenced in these studies (5'UTR) cannot be used to accurately type the viruses that were detected [20–22]. In another study, the

detection of EV-E and -F genomic sequences in RNA extracted from flies, gadflies and cockroaches collected in or near cattle farms led the authors to suggest that some insects may play a role in the transmission of these viruses by passive transport [23]. This hypothesis has never been proven.

The vast majority of EV-Es and -Fs reported in the literature are from stool or rectal swabs, but these viruses have also been detected in nasal swabs and the placenta [9,24]. It is noteworthy that in the dolphin infected with an EV-E, the virus was isolated after swabbing of an erosive lesion of the tongue [11]. EV-Es and -Fs were detected in asymptomatic animals but also in animals with diarrhea [25], enteric or respiratory diseases of varying severity [9,26] and in aborted females [9] but it is difficult to attribute the observed symptoms to EV-Es and -Fs [27]. The experimental infection of twelve calves with an EV-E strain did not reveal any particular clinical signs in infected calves compared to a control group [28].

Very few studies were dedicated to the specific biology of EV-Es and -Fs. They showed that the structure of the EV-E and -F viral particles was similar to that of other EVs [29]. Compared to the EVs found in humans, the main specific genomic trait of EV-Es and -Fs lies within their 5'UTR, which is significantly longer than that of other EVs. It contains about 100 additional nucleotides involved into two secondary structures located between the cloverleaf (domain I) and the IRES: a second cloverleaf (domain I*), and a short stem-loop (domain I**) (Figure 2). Domain I* is not a simple duplication of domain I since these two domains have a relatively low sequence homology with each other. Domain I* sequence significantly differs from that of the cloverleaves observed in the other EV species. *In vitro* studies showed that the replicative capacity of the genome is greatly impaired by the deletion of the domain I** while it is completely abolished by the deletion of the domain I* [30]. In addition, the IRES domains III and VI are significantly shorter in EV-Es and -Fs than in EVs circulating in humans (Figure 2) [31].

Among the known EV-Es and -Fs, two virus types occupy peculiar positions: the EV-E5 and EV-F7 types, each known by a single specimen. Their 5'UTRs belong to the phylogenetic cluster formed by those found in EV-Es and -Fs (Figure 3A) and their non-structural genomic regions are very close to those found in other EV-Es (in the case of EV-E5) [32] or EV-Fs (for EV-F7) [33], but the capsid-encoding regions of these two viruses are relatively distant from those of other known EV-Es and -Fs (Figure 3B), close to the 40% divergence threshold used by the ICTV to define EV species.

Species EV-G

The species EV-G was originally called *Porcine enterovirus* because the first known members of this species were detected in swine. The review of the ancient literature concerning these EVs is complicated by the fact that, for a long time, the term “porcine enterovirus” referred not only to the current EV-Gs but also to viruses now classified in the genera *Teschovirus* and *Sapelovirus* [34]. EV-Gs are classified into 20 types (from EV-G1 to -G20). These viruses have been found in suids (pigs and wild boars), sheep and goats in Europe, Southeast Asia and America. The circulation area and host spectrum of each virus type is impossible to establish given the small number of studies on these viruses. The GenBank database contains a set of partial sequences (GQ250050-GQ250074) of EV-G1s detected in monkeys (*Macaca mulatta*); unfortunately, no publication describes the context in which these sequences were obtained. EV-Gs circulate in pig and sheep farms mainly without noticeable symptoms [35–37]. EV-Gs have been found in animals with diarrhea [37,38] or neurological symptoms, but the role of EV-Gs in these symptoms is difficult to establish given the high prevalence of these viruses in farms [39]. However, in-depth investigations have revealed the presence of EV-G20 genome in the spinal cord of two lambs suffering from limb paralysis [40].

Most known EV-Gs have a 5'UTR whose cloverleaf features a sub-domain D longer than that observed in other EVs [31] (Figure 2). The functional reasons for this extension have not yet been deciphered [41]. A few EV-G specimens have a 5'UTR that is phylogenetically closer to those of EV-Es and -Fs than those of other EV-Gs (Figure 3A). It does not display the extension of the D sub-domain of the other EV-Gs but forms the domains I* and I** found in the EV-Es and -Fs [35]. These specimens are therefore likely descendants of EV-Gs that acquired by recombination a 5'UTR from EV-Es or -Fs (or from unknown viruses close to them in the 5'UTR). The phenotypic consequences of this substitution are not known. It is impossible to date these recombination events or to determine the hosts in which they took place. All known EV-Gs with a 5'UTR close to EV-E and -F ones are from sheep [35,40] or goats, except one specimen detected in an environmental sample [42].

Since 2017, multiple articles have reported the detection in pig farms of EV-Gs of different types whose genomes have an insertion located between the 2C- and the 3A-encoding regions [37,43,44]. The inserted sequence encodes a papain-like cysteine protease that inhibits the immune response of the host [45]. The insertion probably derives from a porcine torovirus and was probably acquired through recombination.

Species EV-I et EV-K

EV-I and -K species are known through a very limited number of genetic sequences and no viruses of these species have been isolated in cell culture to date.

The genomes of EV-I specimens were only identified in one study in stools from four dromedaries [46]. The same study estimated EV-I seroprevalence at 52% in a population of 172 dromedaries. Although phylogenetically distant from EV-Es and -Fs, the genomes detected have a 5'UTR whose organization is similar to that of these viruses (presence of domains I* and I**, domains III and VI relatively short, Figure 2).

EV-Ks were discovered by metagenomics in biological samples of rodents collected in China in 2015 [47]. The genetic distance between the two known EV-K genomes suggests that they belong to two different virus types. Both have a duplication of the cysteine protease domain within the 2A protein (Figure 4) whose role in virus cycle is unknown.

SVDV and SVDV-2, two zoonoses with opposite destinies

The Swine Vesicular Disease Virus (SVDV) was discovered in 1966 in an outbreak affecting two pig farms in Lombardy. Sick animals presented a clinical picture of foot-and-mouth disease; the investigations failed to demonstrate the presence of the Foot-and-Mouth Disease Virus (family *Picornaviridae*, genus *Aphthovirus*) but led to the isolation of the causative agent, the SVDV [48]. The latter showed all the phenotypic characteristics of coxsackieviruses B (CVBs, members of the species EV-B that infect humans) [48], and an obvious antigenic proximity with CVB5s isolated in humans: SVDV is neutralized by antibodies against CVB5s and, reciprocally, anti-SVDV sera neutralize CVB5s [49]. Sequencing of this virus demonstrated that it is indeed a particular CVB5 genetic lineage [50].

Since its initial detection in Italy, SVDV has been involved in outbreaks in pig farms in Europe and Asia. Although they do not cause significant mortality in the farms where they occur, the SVDV outbreaks have a significant impact since they require the implementation of control measures to contain the virus [51].

All known SVDV specimens are from a recent common ancestor, which supports the hypothesis of a single spillover event from humans to pigs, probably in the 1950s or 1960s [52,53]. The available genetic and epidemiological data are too scarce to determine where the host shift took place [54]. SVDV apparently evolves in its new host without any exchange of genetic material with other EVs [52]. The current SVDV strains fall into several genotypes [52] and constitute distinct antigenic variants [55].

As demonstrated by several accidental laboratory-acquired infections, SVDV retains the ability to infect humans [56]. Attempts of experimental infection suggest that the risk of SVDV transmission to other livestock species such as cattle and sheep is low [48,57].

Another case of CVB spillover to pigs was documented retrospectively. It involved a CVB4, which was responsible for epidemics in several pig farms in the Soviet Union in 1975 [58]. The symptoms were identical to those caused by SVDV, but the investigations conducted at the time had revealed the involvement of an EV that antigenically differed from SVDV. Forty years later, the sequencing of a 1975 Soviet isolate demonstrated its genetic proximity to CVB4s circulating in the 1950s and 1960s [58]. Although the transmission of the respective ancestors of SVDV and SVDV-2 from human to swine constitute two independent events, they would therefore be almost contemporary. The SVDV-2 has never been re-observed since. One study experimentally showed its low transmissibility from one animal to another [58], which probably explains why this virus was not able to establish sustained transmission chains in swine and likely disappeared.

Simian EVs

The first EVs specific to NHPs were discovered in the 1950s in biology laboratories handling NHPs or simian cell cultures [59–64]. About ten viruses have been thus identified in different species of NHPs (Table 1). Later, other EVs were identified by isolation or molecular detection in faeces of captive or wild NHPs in Central Africa, Bangladesh, China and the United States [65–71]. The simian EVs have long been named and classified according to a specific taxonomy, which has recently been modified to be consistent with the one applied to the other EVs (Table 1) [72]. The genome organization of the known simian EVs does not differ from that of human EVs [65,73,74].

Simian EVs are now distributed into several species, some of which seem specific to NHPs. Thus, the species EV-J, whose first specimens were identified in the 1950s, now includes six virus types recognized by the ICTV and several specimens belonging to still unnamed types that were recently discovered in NHPs in Central Africa (Table 1, Figure 5A). Two studies of Central African NHPs have identified viruses belonging to a new EV species, which is still unnamed [71,75]; some viruses misidentified as EV-Js in another study [68] actually belong to this new EV species, which includes several virus types of which only two have been named (EV-122 and EV-123). Last, the species EV-H contains only one known virus type (EV-H1), represented by four specimens, while the species EV-L is known only through one specimen [65].

By contrast, some virus types that are seemingly specific to NHPs belong to species that also contain many other virus types that circulate among humans: EV-A, -B and -D (Table 1). The history of these virus types is unknown. Did their ancestors infect humans and were transmitted to NHPs through one or several spillover events? Are the species EV-A, EV-B and EV-D comprised of viruses whose ancestors already infected common ancestors of current primates, including humans? Current data do not allow for these questions to be solved.

Finally, some virus types have been detected both in simian and in human samples, suggesting zoonotic transmission of these viruses. In most cases, the direction of transmission is quite clear. For example, many typically human virus types (including echoviruses, the poliovirus type 3 vaccine strain, and rhinoviruses C) were detected in synanthropic or captive NHPs [10,70,71,76–78]; contacts between NHPs and humans explain the transmission of these human viruses to NHPs. In the opposite direction, the A-2 plaque virus, isolated in the 1960s from an American soldier stationed in Korea [79], is genetically close to the three

strains belonging to a simian EV type, EV-H1 [80]; since this virus has never been re-observed in humans, it is probably a simian virus transmitted occasionally to humans.

Some cases are more puzzling: four virus types (EV-A76, -A89, -A119 and -D111) were detected both in humans and in NHPs living in the dense forest in Central Africa without contact with humans (Figure 6A). It is impossible, based on current data, to formally determine the history of these viruses: are they human viruses transmitted to NHPs despite the apparent geographical remoteness or are they simian EVs that have successfully emerged within the human species? While recombination events continuously reshape the genomic sequences of circulating EVs, it is noteworthy that human and simian specimens of the EV-A76, EV-A89, EV-A90, EV-A91, EV-A119 and EV-A121 types are phylogenetically close to each other across the non-structural genomic region [75,81]: these viruses recombine together but do not exchange genetic material with other EV-As. In addition, their non-structural sequences are phylogenetically closer to those of simian EV-A types (EV-A92, EV-A122, EV-A123 and EV-A124) than those of human EV-As (Figure 6B). Finally, the codon usage bias of these viruses differs from that of the other EV-As and is not optimal for the expression of the viral genome in human cells [82,83]. These observations do not prove that this group of EVs is of simian origin, especially because the types EV-A89, EV-A91 and EV-A121 have never been observed in NHPs; however, this group undoubtedly occupies a peculiar place within the EV-A ecosystem and evolutionary landscape. Moreover, it is striking that all known EV-A119 specimens come from Africa (Figure 6A); this observation is in line with the hypothesis of a simian origin of this virus type, which would have been recently transmitted to humans from NHPs living in Central Africa. A similar observation was made within species EV-D for the type EV-D111, all known specimens of which come from Africa (Figure 6A). The hypothesis of simian origin of EV-D111 is reinforced by the fact that another EV-D

observed in NHPs in Africa, EV-D120, has never been observed in humans, despite the large number of laboratories characterizing EVs in the framework of poliovirus surveillance [84].

Phylogenetic oddities

It is known that recombination events constantly shuffle the EV genomes [6]. The recombination events are limited by several mechanistic and functional factors. The 5'UTR is the most permissive genomic region [85]: genetic exchanges can take place in this region between members of different species (EV-As with EV-Bs, some EV-As with EV-Cs and -Ds, some EV-Gs with EV-Es and -Fs) but all combinations are not possible. The restriction is even more drastic in coding regions where exchanges are possible within the same species but seemingly not between viruses of different species: interspecies recombination is generally not observed in this region (either not occurring or leading to non-functional genomes). In phylogenetic trees, these restrictions can be visualized by the existence of clusters that gather sequences of viruses that are functionally compatible with one another through recombination. For example, whichever the coding region considered, all EV-C sequences fall into a single branch that exclusively contains EV-C sequences (Figure 5B): this indicates that EV-Cs do not exchange coding genetic material with EVs of other species. Across the coding region, this segregation by species is the norm but some simian EVs escape the rule [67,68,71,73,75]: thus, the single known EV-A125 is classified as a member of the species EV-A because its capsid is phylogenetically close to that of other EV-As (Figure 5A), but its non-structural sequence is close to those of members of the species EV-J (Figure 5B); similarly, coding sequences of several types of simian EV-Bs (EV-B110, EV-B112, EV-B113 and EV-B114) are genetically distant from those of human EV-Bs and close to simian sequences belonging to the species still unnamed (Figure 5B). Some simian EV-As and -Bs

thus violate the criteria relative to the degree of intraspecies peptide identity used by the ICTV to define EV species (Boxed text).

This observation may have two explanations: 1- convergent evolution that would have led the non-structural sequences of EVs of different species to approach each other phylogenetically. For example, it could be an adaptation to replication in NHPs. However, it should be noted that some typically simian EV-As (EV-A92, EV-A122, EV-A123 and EV-A124) are phylogenetically close to human EV-As in all genomic regions [69]; so, they would not have been subjected to this mechanism of convergent evolution, which would not affect the simian viruses belonging to the species EV-D, EV-H and EV-L either; 2- recombination events between simian EVs of different species. This would be an uncommon genetic plasticity since, as noted above, interspecies exchanges seem impossible between EVs in the coding genomic regions. It is nevertheless possible that our understanding of the restrictions that apply to recombination between EVs is biased by the fact that it is essentially based on observations made among EVs circulating in humans. *In vitro* studies have shown that genetic recombination between EVs is capable of producing a wide variety of mosaic genomes, most of which are eliminated through counter-selection [86–89]. One of the restrictions that limit recombination is the association within a single molecule of genomic regions not being functionally compatible with one another: this type of association generates non-functional genomes that are unable to generate infectious viral particles [90]. It is therefore certain that functional incompatibilities limit recombination between phylogenetically distant EVs, and particularly between EVs belonging to different species. It is possible, however, that, for unknown reasons, the constraints on recombinant genomes may be looser in animal EVs than in human EVs, thus making interspecies recombination much more common in EVs circulating in animals. [90].

Conclusion

Our knowledge about EVs circulating in animals is fragmentary. It is all the more limited as the majority of known animal EVs have been found in domestic animals; the exploration of EVs circulating in wildlife is still to be done. Many questions remain open: what is the respective host spectrum of each EV species? What are the factors that define the host spectrum? What is the geographic area of each EV species? What are the symptoms associated with each species and virus type?

Despite the small number of studies on these viruses, a wide variety of EV species and types have already been identified in animals, often from small samples. Although relatively few in number, animal EV specimens reveal the existence of notable characteristics: organization of the region 5'UTR that varies from one species to another, duplication of an enzymatic region in EV-Ks, unusual phylogenetic relationships between certain EVs. Each of these observations could be the starting point for a new research project.

Another aspect of studying animal EVs is the existence of zoonotic transmission. Again, there are many questions to be addressed. How common are zoonotic infections? What are the factors that allow spillover events? Are some human EVs the descendants of animal EVs that have managed to adapt to humans? For example, it was suggested that EV-D70, which has been responsible for epidemics of haemorrhagic conjunctivitis in humans since the late 1960s, has a zoonotic origin [91].

Thus, the study of animal EVs constitutes a vast field of research with many interests: it relates to viruses that may have significant economic repercussions by infecting livestock and that could have huge impacts on human health in case of spillover events. In addition, the study of viruses phylogenetically distant from the most studied EVs should reveal the existence of unsuspected biological processes.

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ICTV's species demarcation criteria within the genus *Enterovirus*

Two EVs belong to the same species if:

- 1- They share a common genome organization;
- 2- They are less than 40% divergent in capsid nucleotidic sequence;
- 3- They are less than 30% divergent in polyprotein peptidic sequence;
- 4- They are less than 30% divergent in peptidic sequence of the non-structural proteins 2C + 3CD.

Figure 1. Canonical organization of the EV genome. The 5' genome extremity is covalently linked to a virus protein, VPg; the 3' extremity is polyadenylated. The 5'UTR is folded into secondary structures (domains I to VI or I to VII, depending on the viruses). The domain I is a cloverleaf involved in the replication of the genome; the other domains together form the internal ribosome entry site, which allows the translation of the genome in a cap-independent manner. The typing of EVs mainly relies on the region that encodes the VP1 capsid protein.

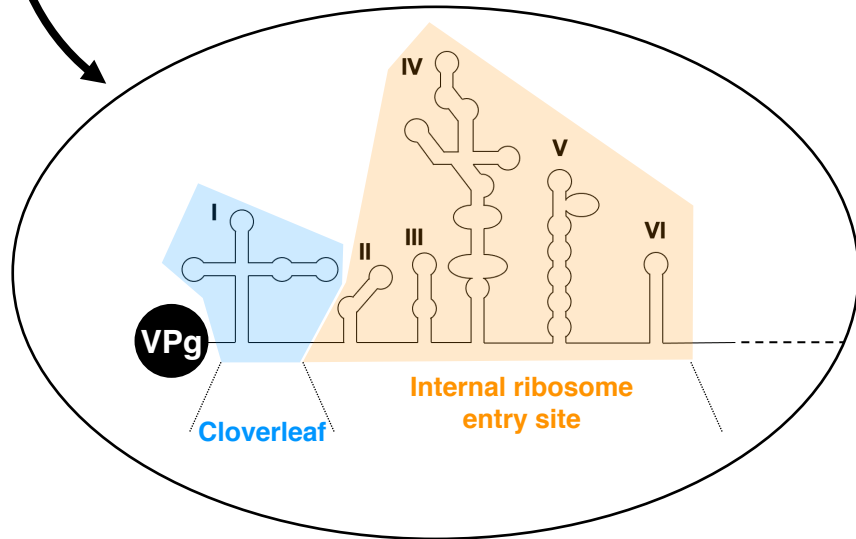
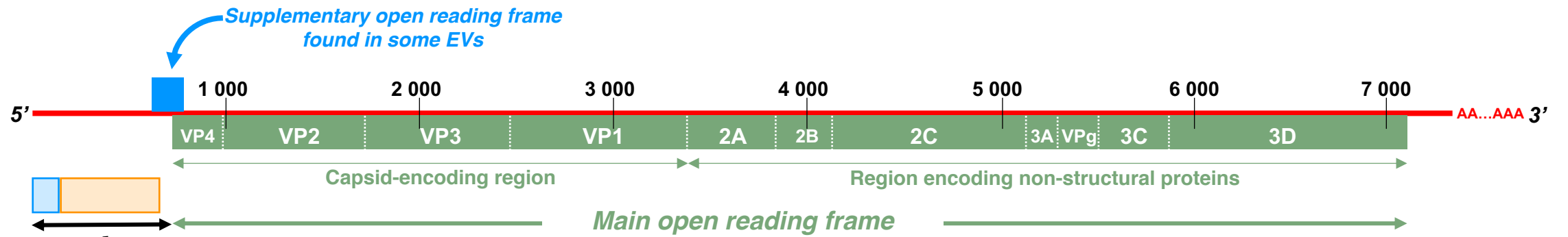
Figure 2. Secondary structures found in the 5'UTR of different EV species.

Figure 3. Phylogenetic relationships between EV-Es, EV-Fs and EV-Gs in two genomic regions: the 5'UTR (A) and the VP1-encoding region (B). Asterisks indicate EV-G specimens that have 5'UTR closely related to those of EV-Es and EV-Fs. The dendograms were drawn using the neighbour-joining method with MEGA X.

Figure 4. Protease peptidic sequence found duplicated in the EV-K 2A protein. The peptidic sequences of the two known EV-Ks are aligned with two copies of the 2A protease domain of an EV-A71. The residues involved in the catalytic triad are in red, the residues linked to a zinc atom are in blue. The asterisks indicate the conserved residues.

Figure 5. A. Phylogenetic relationships of the VP1-encoding regions of different EV types belonging to EV species that contain simian EVs. Each virus type is represented by one specimen. The virus types that are seemingly specific to non-human primates are indicated by coloured circles. B. Phylogenetic relationships of the 3D polymerase-encoding regions. This dendogram highlights that some simian virus types belonging to the species EV-A and EV-B are, in this region of the genome as in the whole non-structural region, closer to simian types belonging to other species than to virus types belonging to their respective species. Representatives of types that are still unnamed are indicated by their respective Genbank accession numbers. The dendograms were drawn using the neighbour-joining method with MEGA X.

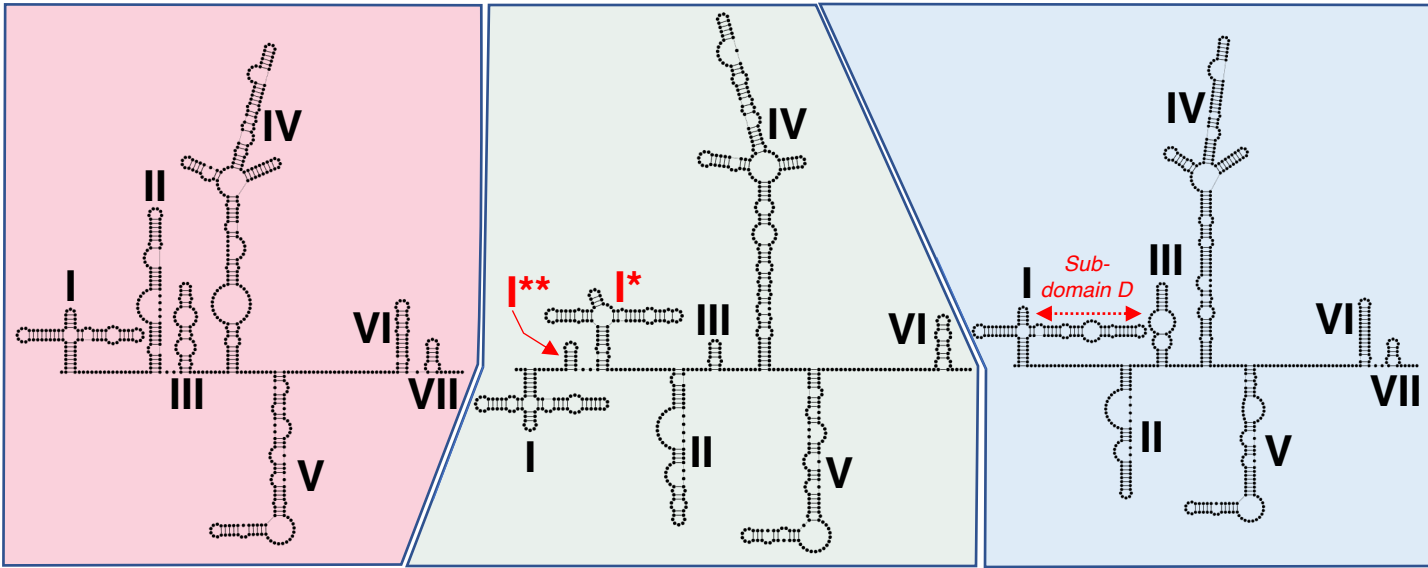
Figure 6. A. Phylogenetic relationships between specimens of four virus types that contain both human specimens and specimens coming from non-human primates living with minimal contacts with humans (VP1-encoding region). B. Phylogenetic relationships between EV-As in the 3D polymerase-encoding region. This dendogram shows that the types EV-A92/EV-A122/EV-A123/EV-A124 on the one hand and EV-A76/EV-A89/EV-A90/EV-A91/EV-A119/EV-A121 on the other hand form, in this region, two distinct clusters. The sequence of an EV-B (CVB3) was used to root the tree. The dendograms were drawn using the neighbour-joining method with MEGA X.

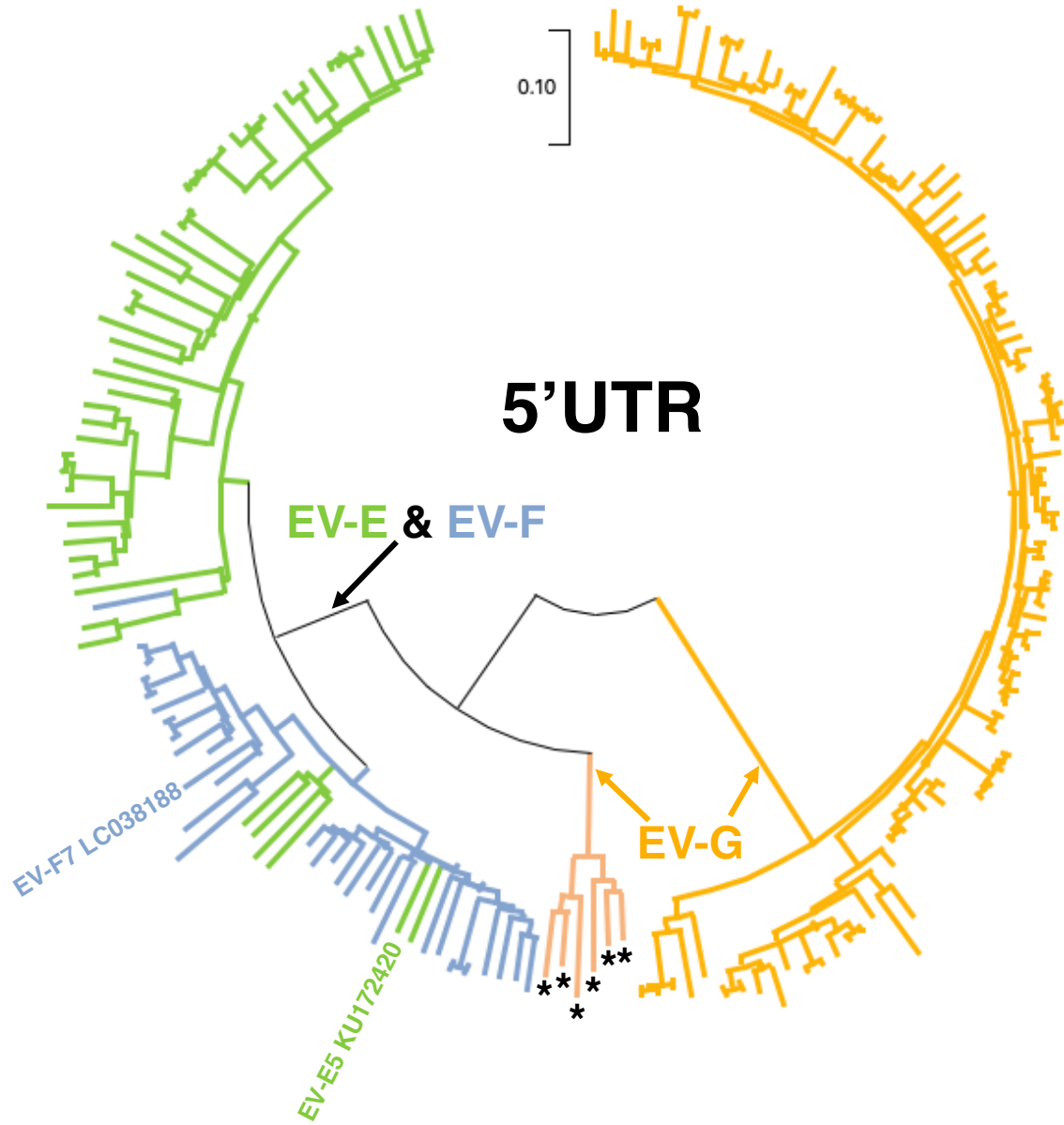
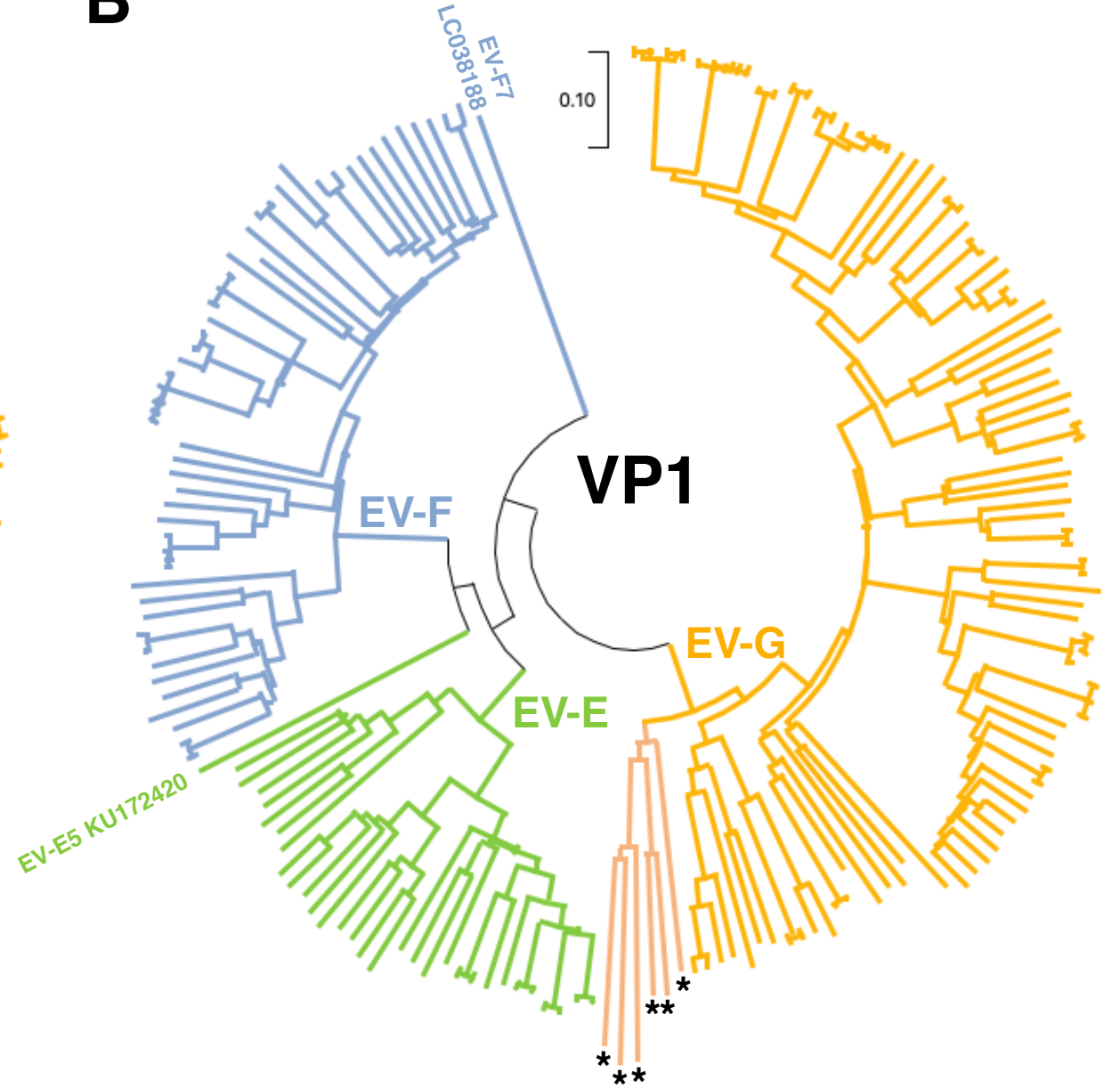


EV-As, EV-Bs, EV-Cs,
EV-Ds, EV-Js, EV-Ls

EV-Es, EV-Fs, EV-Ia,
some EV-Gs

Most known EV-Gs



A**B**

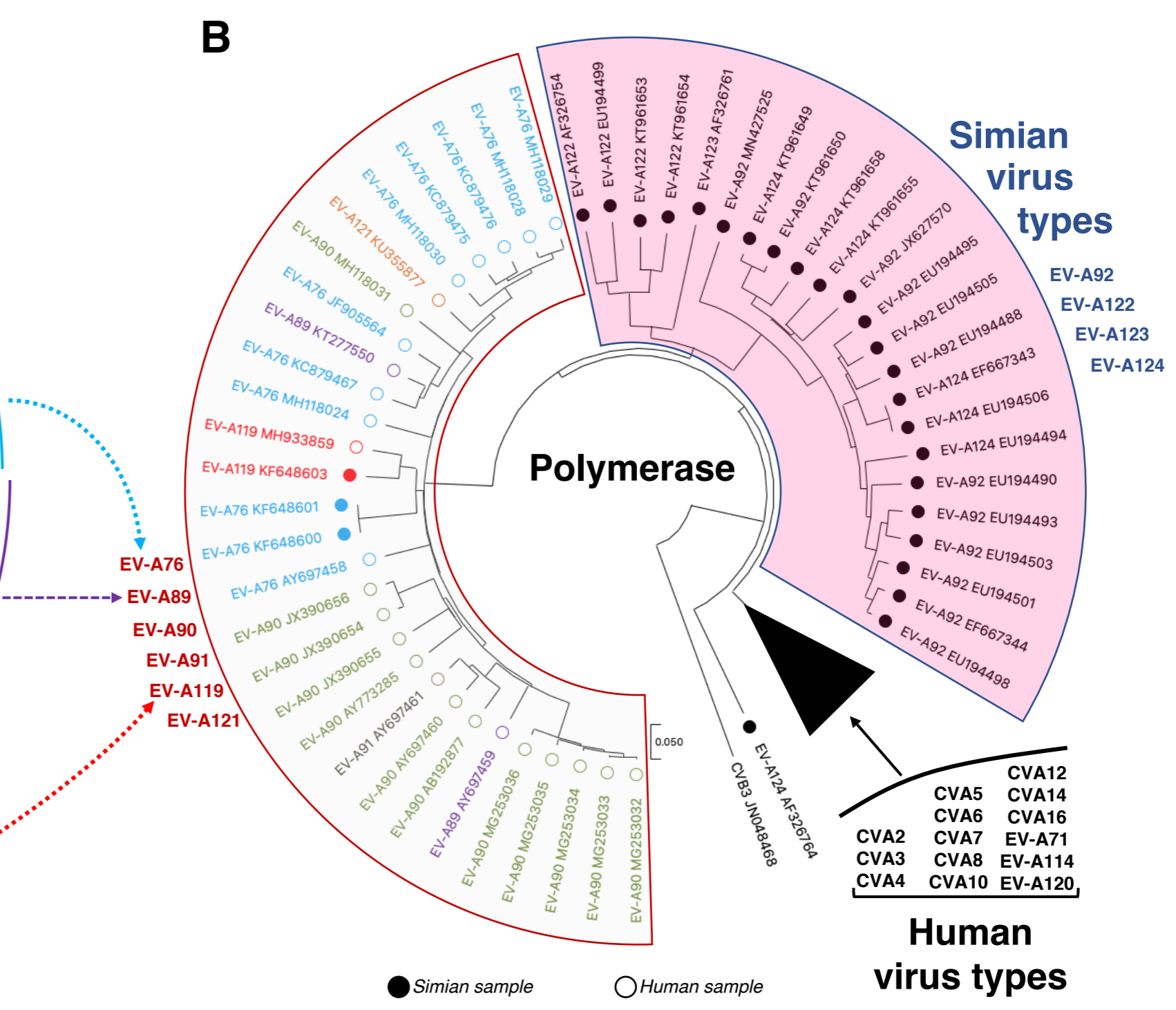
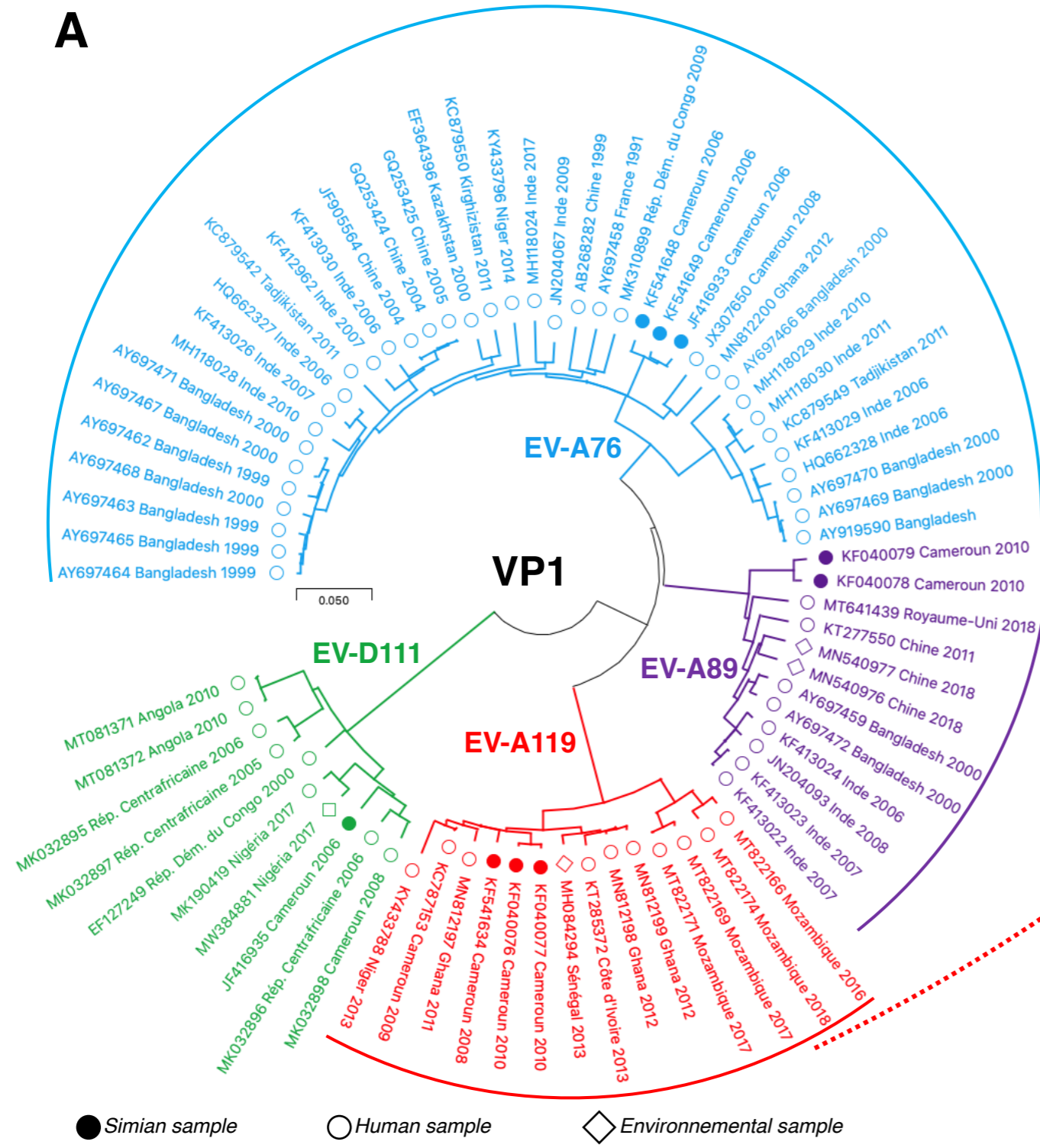
EV-A71 GKFQ~~Q~~SGAIYVGNFRV~~VNR~~HLATHTDWANLVW-EDSSRDLLVSSTTAQ-GCDTI--ARCNCQTGVYYCNSRRKHYPVS
KX156158 SWLGDNSGVV-AGSFLYINKHIAP~~T~~SMLQHA-KFDSDEL~~D~~LQIYRVESQHEQNNVDIPQCTCGKGWYYSGLTRHVQHAL
KX156159 SWFGDKQGFV-AGNKYYVNYHLVPPHMLKHATKIDKFH-DLCIFEVKEQDARPEVQIPRCTCCKGWYYSNLTRRIQHAY
* * * * * ** * * * * *

EV-A71 FSKPSLVFVEASEEYYPARYQSHLMLAEGHSEPGDCGGILRCQHGVVGVSTGGS--GLVGFADVRDLLWLDEEAMEQ
KX156158 LTPPVVQHFNAGEFYPEHYQI-VHLAEGPGMGDCGNVLYCRHGPIGLLT-G-SADGIVAFIPLRIFVGMFKEEYNAAL
KX156159 ISPPRMQHF~~E~~ASQWYPH~~F~~QE-VHLAEGPGQEGDCGNVLYCRHGPIGMVT-G-AARGIVAFIPLRIWAGAFLEHMAVI
* * * * * **** * * * * * * * * * *

EV-A71 GKF-GQQSGAIYVGNFRV~~VNR~~HLATHTDWANLVW-EDSSRDLLVSS
KX156158 A-----EIRALN-----KPEKPSFKD-RIL---SFFSRGQ-QGCTVGIHQIKPRHLTTPLD---KVVEDHPPERDITVVL
KX156159 KYALDNDICTVDGTDVWKGKGEWKVFRNL~~D~~ERSFKTHGKTRGIAIGAVQLIPAH-THQGE---RVLAHHGSR~~E~~VLVVE
* * * * *

EV-A71 TTAQ-GCDTI--ARCNCQTGVYYCNSRRKHYPVSFSKPSLVFVEASEEYYPARYQSHLMLAEGHSEPGDCGGILRCQHGV
KX156158 APQNPEHKI--AGCDLQGCYSSATGRYHSIDL~~C~~APSFRLISG--PYGEQYVSHVMTGEGVAQPGD~~S~~GQPILCQHGI
KX156159 G--RPGCHALTPAVCDCHSGIYYSHKTTRIHHVQLSPPGLYKFRT--PEGERYRSSVMVGC~~G~~PAQPGD~~C~~GQPIFCGHGV
* * * * * * * * * * * * * * *

EV-A71 VGVSTGGS--GLVGFADVRDLLWLDEEAMEQ
KX156158 FGLLIAGDTQKHTVSFADIRDLAPRYQ-IYSQ
KX156159 FGMLIGGDEALGTVAFADVRDLMARYV-AREQ
* * * * * * * * *



CVA12	CVA14
CVA5	CVA16
CVA6	EV-A71
CVA2	CVA7
CVA3	CVA8
CVA4	CVA10
	EV-A114
	EV-A120

Table 1. EV virus types identified in non-human primates.

Current name	Former names	Collection year of the first specimen [bibliographic reference]	Known host species
EV-A			
EV-A92		1999 [67]	<i>Macaca mulatta</i> , <i>M. nemestrina</i>
EV-A122	P2, SV19	1950s [59]	<i>Macaca sp.</i> , <i>Pan troglodytes</i>
EV-A123	P12, SV43	1950s [59]	<i>Macaca fascicularis</i>
EV-A124	P15, SV46	1960s [59]	<i>Macaca sp.</i>
EV-A125	BaEv, BA13, SEV-18	1960s [57]	<i>Papio anubis</i> , <i>P. hamadryas</i>
EV-B			
EV-B110		2006 [65]	<i>Pan troglodytes</i> , <i>Mandrillus sphinx</i>
EV-B112		2009 [66]	<i>Pan troglodytes</i>
EV-B113		2010 [66]	<i>Mandrillus sphinx</i>
EV-B114	SEV-17, SA5	1950s [61]	<i>Cercopithecus aethiops</i> , <i>Mandrillus sphinx</i>
EV-D			
EV-D120		2010 [64]	<i>Gorilla gorilla</i> , <i>Pan troglodytes</i>
EV-H			
EV-H1	SA4, SV4, SV28, A-2 plaque, SEV-16, SEV-A1	1950s [60]	<i>Cercopithecus aethiops</i> , <i>Macaca mulatta</i>
EV-J			
EV-J103	EV103	1999 [67]	<i>Macaca mulatta</i> , <i>M. nemestrina</i>
EV-J108	SEV-N125 & SEV-N203	1970s [62]	<i>Papio cynocephalus</i>
EV-J112		2008 [68]	<i>Macaca mulatta</i> , <i>Papio anubis</i> , <i>P. hamadryas</i>
EV-J115		2007 [68]	<i>Cercocebus chrysogaster</i> , <i>Macaca mulatta</i>
EV-J121		2006-2008 [69]	<i>Pan troglodytes</i>
EV-J122	SV6	1950s [60]	<i>Macaca mulatta</i>
Several unnamed virus types		2018 [Li et al. <i>Infect Genet Evol.</i> (2020) 82:104279]	<i>Chlorocebus sabaeus</i>
EV-L			
EV-L1	SEV-Gx	2014 [63]	<i>Macaca mulatta</i>
Unnamed species			
EV-122		2006-2008 [69]	<i>Cercocebus torquatus</i>
EV-123		2006-2008 [69]	<i>Cercocebus torquatus</i> , <i>Mandrillus sphinx</i>
Several unnamed virus types		2007-2011 [73, 75]	<i>Mandrillus sphinx</i>