# Retroviral Diversity and Distribution in Vertebrates

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We used the PCR to screen for the presence of endogenous retroviruses within the genomes of 18 vertebrate orders across eight classes, concentrating on reptilian, amphibian, and piscine hosts. Thirty novel retroviral sequences were isolated and characterized by sequencing approximately 1 kb of their encoded protease and reverse transcriptase genes. Isolation of novel viruses from so many disparate hosts suggests that retroviruses are likely to be ubiquitous within all but the most basal vertebrate classes and, furthermore, gives a good indication of the overall retroviral diversity within vertebrates. Phylogenetic analysis demonstrated that viruses clustering with (but not necessarily closely related to) the spumaviruses and murine leukemia viruses are widespread and abundant in vertebrate genomes. In contrast, we were unable to identify any viruses from hosts outside of mammals and birds which grouped with the other five currently recognized retroviral genera: the lentiviruses, human T-cell leukemia-related viruses, avian leukemia virus-related retroviruses, type D retroviruses, and mammalian type B retroviruses. There was also some indication that viruses isolated from individual vertebrate classes tended to cluster together in phylogenetic reconstructions. This implies that the horizontal transmission of at least some retroviruses, between some vertebrate classes, occurs relatively infrequently. It is likely that many of the retroviral sequences described here are distinct enough from those of previously characterized viruses to represent novel retroviral genera.

Vertebrate genomes contain numerous parasitic genetic elements, many of which undergo vertical germ line transmission and are capable of remaining in the same locus for millions of years (40, 45). Some of the best studied of these elements are members of the Retroviridae which, as exogenous infectious viruses, cause neurological and immunological diseases, malignancies, and immunodeficiencies (4). Prior to integration within their host's genome, retroviruses use virally encoded reverse transcriptase to copy their RNA genome into DNA. The low fidelity of this enzyme results in a high rate of mutation, with the subsequent result that retroviruses have highly divergent nucleotide sequences (36). However, there are regions of the retroviral genome, especially within the polymerase gene, which are reasonably well conserved when different isolates are compared, and this has enabled phylogenetic trees of the retroviruses to be constructed (6, 8, 45). The latest classification, based at least partly on this type of analysis, placed retroviruses into seven genera: the spumaviruses, murine leukemia-related viruses (MLVs), lentiviruses, human Tcell leukemia-related viruses (HTLVs), avian leukosis viruses (ALVs), type D viruses, and mammalian type B viruses (4, 9). With few exceptions, all of the retroviruses from these genera were isolated from mammalian (and a small number of avian) hosts, leaving questions pertaining to their origin, evolution, and distribution within other vertebrates that remain largely unanswered. Other questions of interest concern the host range boundary of the Retroviridae and whether additional retroviral genera remain to be discovered. With regard to the latter point, several recently reported novel retroviruses in reptiles, amphibians, and fish have suggested that this may indeed be the case (13, 15, 39). One approach to answering these questions involves the

amplification of endogenous retroviral sequences by PCR (34, 43), as there are several highly conserved motifs within retroviral proteins against which degenerate oligonucleotide primers can be designed (37, 41, 45). It should therefore be possible to obtain a good idea of the retroviral diversity within a range of organisms by using this methodology, in conjunction with multiple phylogenetic analyses of the resultant sequences.

Here we use this procedure to isolate and characterize novel endogenous retroviral sequences from a wide range of vertebrate hosts and to examine their relationships with previously described retroviruses.

## MATERIALS AND METHODS

**Nomenclature.** The term retrovirus is currently used to describe two different (but overlapping) sets of retroelements: (i) a member of the *Retroviridae*, in the sense that this family is monophyletic with respect to other retroelements, and (ii) an infectious retroelement, in the sense that (in addition to many members of the *Retroviridae*) the gypsy long terminal repeat (LTR) retrotransposon in *Drosophila melanogaster* can also be transmitted horizontally (16). For purposes of clarity we use the former definition in this report.

Sequencing and sequence analysis. Amplification products were electrophoresed through 1.3% agarose gels, and products of between 600 and 1,200 bp were excised, purified, and cloned into the vector pCRII (Invitrogen). Cloned inserts were sequenced in both directions, either manually using a Sequenase kit (U.S. Biochemicals) or with an automated DNA sequencer (ABI 373 Stretch) and a

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dye-terminator kit (Perkin-Elmer Cetus). Retroviral sequences were initially identified by BLAST searches (1) or by screening CD-ROM sequence libraries.

Phylogenetic analysis. The data matrix consisted of 190 amino acid residues, 160 derived from the reverse transcriptase protein (aligned as described in reference 45) and 30 from the protease [from 15 residues 5' to 12 residues 3' of the well-conserved GR(D/N) motif]. Phylogenetic trees were constructed by using the program PAUP4d-56-4d61 (written by D. L. Swofford) and utilized both the neighbor-joining and maximum parsimony approaches. All trees, except where stated, were generated by using amino acids and an unordered matrix. Six equally parsimonious trees were identified after the data set was subjected to a heuristic search comprising 100 random-addition replicates. The robustness of each node was assessed by bootstrap resampling with 1,000 replicates using neighbor joining or with 100 replicates using maximum parsimony (each of 25 random-addition sequences with all characters unordered). The group III sequences were investigated further by using a longer sequence alignment of 257 amino acid residues. The phylogeny of these sequences was reconstructed using the PROTPARS matrix and was subjected to 1,000 bootstrap replicates with neighbor-joining analyses or 100 bootstrap replicates (each with five random additions) in the case of maximum parsimony. The level of clustering of the group III sequences into clades derived from a single vertebrate class was examined by using the program MacClade (20). A multistate character representing the host class was scored for each taxon, and the minimum number of character state changes (i.e., shifts between host classes) required by the phylogeny was then calculated. This number was then compared to those obtained for each of 100 test replicates in which the host class characters were shuffled randomly between the viral taxa while maintaining the same tree. A smaller number of steps required to generate the fit of the real associations, when compared to the fit of the random associations, indicates that the virus-host associations are phylogenetically correlated.

Southern hybridization. Southern hybridization analysis was performed by the method of Sambrook et al. (32). Hybridization of each of the fragments to 10  $\mu$ g of host genomic DNA was carried out at 65°C, and the filters were washed down to 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% sodium dodecyl sulfate at 65°C.

Sequence sources. Sequences were obtained from sequence databases with the original sources as described in a previous report (38), in the text, or as follows: micropia (18), feline syncytial foamy virus (FeSFV) (14), human endogenous retrovirus HERV.L (5), murine endogenous retrovirus MuERV.L (2), endogenous retrovirus type 9 (ERV-9) (17), HERV.I (21), human cosmid U85A3 [Hcosmid(U85A3)] (25), jaagsiekte (46), HERV.K10 (26), and RSV (33). The sequences described here have been submitted to the EMBL/GenBank/DDLB databases with the following accession numbers: RV-common possum, AJ225211; RV-stripe faced dunnartI, AJ225230; RV-stripe faced dunnartII, AJ225231; RVbower bird, AJ225208; RV-tinamou, AJ225235; RV-pit viper, AJ225222; RVtuatara, AJ225236; RV-gharial, AJ225215; RV-slider turtleI, AJ225227; RVslider turtleII, AJ225228; RV-palmate newtI, AJ225220; RV-palmate newtII, AJ225221; RV-tiger salamanderI, AJ225232; RV-tiger salamanderII, AJ225233; RV-tiger salamanderIII, AJ225234; RV-rhinatremid caecilianI, AJ225224; RVrhinatremid caecilianII, AJ225225; RV-rocket frog, AJ225226; RV-edible frog, AJ225212; RV-leopard frog, AJ225218; RV-Iberian frog, AJ225217; RV-European frog, AJ225213; RV-African clawed toad, AJ225207; RV-painted frog, AJ225219; RV-stickleback, AJ225229; RV-brook trout, AJ225209; RV-brown trout, AJ225210; RV-freshwater houting, AJ225214; RV-puffer fish, AJ225223.

### RESULTS

Retroviruses have highly variable nucleotide sequences (6, 45), and therefore, to ensure PCR amplification of as wide a range of retroviral sequences as possible, several degenerate oligonucleotide primers were used in this study. Table 1 shows the amino acid motifs on which the primers were based and those retroviral genera which they were predicted to amplify. The PRO primer, based on the protease protein, was used in combination with either CT, JO, or EM, which are complementary to the conserved motif within domain 5 of the reverse transcriptase protein. Virtually all described retroviruses encode either YVDD or YMDD in this position (Table 1), and thus, together, the reverse transcriptase primers are suitable for amplifying retroviruses from all seven genera. This is also the case for the protease primer, as all known retroviral proteases encode the motifs DTGA or DSGA (41), against which the PRO primer was designed. Furthermore, because many elements from a separate retroelement family, the gypsy LTR retrotransposons, also contain identical or very similar sequences, it seemed probable that even highly divergent retroviruses would be amplified by a PCR-based approach utilizing

TABLE 1. Oligonucleotide primer motifs and target genera

Primer	Target site	Target genera
PRO <sup>a</sup>	5'V(F/L)(F/L/V)DTG(S/A)3' (protease)	All
$\mathrm{JO}^a$	5'ŶVDD(L/I/M)LI3'(RT) <sup>b</sup>	Spumaviruses MLV-related viruses
CT EM	5'(H/Q)YVDDLL3'(RT) 5'YMDD(L/I/M)L(I/M)3'(RT)	MLV-related viruses HTLV-BLV group
		ALV-related viruses Type D viruses Mammalian type B viruses
		Lentiviruses

<sup>a</sup> PRO and JO are also capable of amplifying gypsy and copia LTR retrotransposons.

<sup>b</sup> RT, reverse transcriptase.

these primers. Although CT, JO, and EM were designed to amplify specific retroviral subgroups or genera (Table 1), some cross-amplification of nontarget genera was observed. Table 2 shows the reverse transcriptase primer used in each successful amplification, the host species, and the name of the isolate.

PCRs were performed on genomic DNA samples from more than 50 taxa (which were either obtained from other researchers or prepared from tissue samples). The taxa included members of eight vertebrate and three nonvertebrate classes (Fig. 1), but were mainly derived from reptiles, amphibians, and bony fish, as there have been numerous reports of retroviral particles in these organisms (24, 30). Retroviral sequences were identified in each of the vertebrate classes surveyed, with the exception of the lampreys (Cephalaspidomorphi, such as the river lamprey Lampetra fluviatilis) and the hagfish (Myxini, such as the Taiwanease hagfish Myxine yangi). Furthermore, no retroviral sequences were amplified from nonvertebrate hosts: we examined the lancelet Amphioxus floridae (Cephalochordata), the sea squirt Ciona intestinalis (Urochordata: Ascidiacea), and nine species of mollusc (including the soft-shelled clam Mya arenaria). Almost all the amplifications were performed in duplicate, using both the CT and JO primers, whereas the EM primer was used (generally in parallel with the other primers) with a somewhat smaller number (approximately 40) of taxa.

Amplification often resulted in the isolation of more than one clone with homology to reverse transcriptase. However, these clones were usually very similar or identical to each

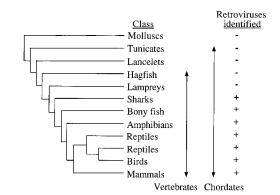


FIG. 1. Distribution of retroviral sequences within vertebrates and other Metazoa. The phylogeny is based on that described by Young (47). Endogenous retroviral sequences identified by PCR screening are indicated by a +, whereas a - represents screened taxa from which retroviral sequences have yet to be identified.

TABLE 2. Retroviral isolates from which sequence data is available, excluding those derived from placental mammal	TABLE 2. Retrove	iral isolates from which	n sequence data is availa	able, excluding those	derived from placental mammals
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darampia's Common presum (Trichourner vulpecula) Stripe-faced damant (Sminitopsis macrouro) EM RV-stripe faced damant Rv-stripe faced dam	Host organism	Primer	Name <sup>a</sup>
Stripe-faced dumart (Smithdopsis macroura)       EM       RV-stripe faced dumart RV-stripe faced dumart (T       RV-stripe faced dumart RV-stripe faced dumart (T         Rock wallaby (Perragale gadmam)       CT       RV-rock wallaby (21)         Wirk       Chickon (Galifiormes Dack       SV (3)         Chickon (Galifiormes Dack       SV (41)       ENV (30)         Order Paseriforms       BU       RV-sparrow (21)         House sparrow (Pass domenticus)       JO       RV-sparrow (21)         Regent bower bid (Sciencha backer)       EM       RV-sparrow (21)         Order Tasseriforms       JO       RV-sparrow (21)         South American pit viper (Boltrops jarrucu)       JO       RV-tinamou         South American pit viper (Boltrops jarrucu)       JO       RV-vinatra         South American pit viper (Boltrops jarrucu)       JO       RV-vinatra         Order Caccodylia       CT       RV-formato diargon (21)         Charial (Garial gangetica)       CT       RV-formato diargon (21)         Order Tuberodia bagenetica)       JO       RV-stript lace         Order Caccodylia       CT       RV-shainatl         Gharial (Garial gangetica)       JO       RV-shainating         Order Tuberodia lagongtica       JO       RV-shainatra         Order Caccodyl	Marsupials		
Cr     RV-stripc faced dumanti       Sinds     Cr     RV-rock wallaby (21)       Order Galiformes     SVV (41)     RV (32)       Dack     Order Transition     SVV (41)       House sparrow (21) Regent bower bird (Scientus bakeri)     JO     RV-tinamou       Order Tyamintores     I     RV-tinamou       Elegant crested finamou (Eudomia degant)     JO     RV-tinamou       Order Synamatic (onakes and lizards)     South American pix typer (Rotrog literarca)     JO       South American pix typer (Rotrog literarca)     JO     RV-timenou       Cr     RV-splatiall     Order Synamatic (onakes and lizards)       South American pix typer (Rotrog literarca)     JO     RV-timenou       Cr     RV-splatiall     Order Synchotensplatin (utarta)       Totata (Spletondon sp.)     JO     RV-splatiall       Order Cocoolylia     Cr     RV-splatiall       Gradi (Gandaig angerica)     Gr     RV-splatial utalcal       Order Cocoolylia     Cr     RV-splatiall       Order Cocoolylia     Gradie turbe (Chrosensy scripta)     JO       Ord			
Rock wallaby (Petrogale godmani)     CT     RV-rock wallaby (21)       Sinds     Order (sufficiences)     RV-rock wallaby (21)       Order Assertion     RV (21)     RV (20)       Darks (Molegre gulgapoue)     LDV (11), REV (30)     DV (11), REV (30)       Darks     SNV (41)     RV-sparrow (21)       Regent bower foil (Sericulus backri)     EM     RV-sparrow (21)       Regent bower foil (Sericulus backri)     EM     RV-sparrow (21)       South American pit viper (Bottrops jarrarca)     JO     RV-tinamou       South American pit viper (Bottrops jarrarca)     JO     RV-tinamou       South American pit viper (Bottrops jarrarca)     JO     RV-tower bid       Order Caccoblia     CT     RV-konsold origon (21)       Gharini (Grandir generation)     JO     RV-staili       Order Caccoblia     CT     RV-staili       Gharini (Grandir generation)     JO     RV-stailer turtlel       Apphibins     JO     RV-stailer turtlel       Order Caccoblia     Granmader in an ewits)     FM       Palmate newi (Grans and newis)     JO     RV-stailer turtlel       Palmate newi (Grans and newis)     FM     RV-spalmate newil       Tager salamander (Grans and newis)     JO     RV-triger salamander!!       Order Consunoptiona (caccilian)     CT     RV-triger salama	Stripe-faced dunnart (Sminthopsis macroura)		
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Regent bower bird (Scriculus bakeri)     EM     RV-bower bird       Order Tnamiformes     JO     RV-inamou       Elegant crested finamou (Eudronia elegans)     JO     RV-intamou       South American pir viper (Bohrnys forunca)     JO     RV-pit viper       South American pir viper (Bohrnys forunca)     JO     RV-pit viper       Komodo dragon (Varaus konodoensis)     CT     RV-Komodo dragon (21)       Order Rhynchocephalia (tuatara)     JO     SpeV (38)       Tuatara (Sphenodon sp.)     JO     RV-shider turtlel       Order Checiona (untles)     T     RV-shider turtlel       Order Checiona (untles)     JO     RV-shider turtlel       Inphibians     Order RV-palmate newtl     Suffer turtlel       Order Anura (Ambystona tigrinum)     JO     RV-palmate newtl       Tiger salamander (Ambystona tigrinum)     JO     RV-riagre salamanderII       Order Anura (frogs and toads)     CT     RV-rimatremid caecilian       Amazonian poison dart frog (Dendrobates ventrimaculatus)     JO     Devil (37)       JO     RV-redicte (shawed toagens)     GT     RV-rimatremid caecilian       CT     RV-rimatremid caecilian     JO     Devil (37)       Order Anura (frogs and toads)     GT     RV-rimatremid caecilian       Amazonian poison dart frog (Dendrobates ventrimaculatus)     JO		IO	RV-sparrow (21)
Order Tinamiformes       JO       RV-tinamou         Elegant crested tinamou (Eudomia elegans)       JO       RV-tinamou         Order Squanata (snakes and lizards)       South American pit viper (Bohrops jararca)       JO       RV-pit viper         South American pit viper (Bohrops jararca)       JO       SpeV (38)       CT       RV-konodo dragon (21)         Order Rynchocephalia (tutara)       Tutatra (Sphenodon sp.)       JO       SpeV (38)       CT       RV-tutatra         Order Crocodyla       CT       RV-stafar III       Gtafar (Gravials gangeticus)       CT       RV-stafar III         Order Crocodyla       JO       RV-sider turtle!       NV-stafar III       SpeV (38)         Order Crocodyla       JO       RV-sider turtle!       NV-stafar III       SpeV (38)         Order Crocodyla       JO       RV-sider turtle!       NV-sider turtle!         Minibians       JO       RV-palmate newt!       Steve (38)         Order Gravinophiona (cacilians)       JO       RV-palmate newt!       RV-sider stafamader!!         Order Gravinophiona (caccilians)       CT       RV-finamerimid caccilian       RV-finamerimid caccilian         Order Gravinophiona (caccilians)       CT       RV-sider turtle!       RV-sider turtle!         Order Gravinophiona forg (Ama tagintum)			
Elegant crested tinamou (Eudromia elegans)       JO       RV-tinamou         Reptiles       Order Squamata (snakes and lizards)       JO       RV-pit viper         South American pit viper (Bolhrops jaranzaa)       JO       RV-pit viper         Komodo dragon (Varanus komodoensis)       CT       RV-Komodo dragon (21)         Order Rhynchocephalia (tuatara)       JO       SpeV (38)         Tuatara (Sphenolon sp.)       JO       RV-stinatara         Order Choina (turtles)       JO       RV-stinatara         Order Choina (turtles)       JO       RV-slider turtleI         Naphibians       JO       RV-pitate newII         Order Choina (turtles)       JO       RV-palmate newII         Tiger salamander (Ambystoma tigrinum)       JO       RV-palmate newII         Tiger salamander (Ambystoma tigrinum)       JO       RV-tiqer salamanderII         Order Choina (cacellians)       CT       RV-tiqer salamanderII         Rhinatternid cacellian (Epictenops marmoratus)       CT       RV-timatrenid cacellian         Order Symophiona (cacellians)       IO       DevI (37)         Amazonian poison dart frog (Dendrobates ventrimaculatus)       JO       DevII (37)         JO       RV-teorokat frog       RV-tockat frog         European conmon frog (Raua temporari		2002	
Örder Squamata (snakes and lizards)         South American pit viper (Bohrops jamaca)       JO       RV-pit viper         Komodo dragon (Varanus komodoensis)       CT       RV-komodo dragon (21)         Tuatara (Sphenodon sp.)       JO       Spec (38)         Order Crocodylia       CT       RV-tuatara         Gharial (Gavalia gangeticus)       CT       RV-gharialII         Order Crocodylia       JO       RV-silder turtleI         Red-eared slider turtle (Chrysenys scripta)       JO       RV-silder turtleI         Jophilians       JO       RV-palmate newtI         Order Urodela (salamanders and newts)       EM       RV-palmate newtI         Palmate newt ( <i>Triturus helveticus</i> )       JO       RV-tiger salamanderIII         Tiger salamander (Ambystoma tigrinum)       CT       RV-tiger salamanderIII         Order Anura (frogs and toads)       CT       RV-thinatremid caecilian         Amazonian poison dart frog (Dendrobates ventrimaculatus)       JO       DevII (37)         JO       RV-defined       JO       RV-splande frog         Leopard frog (Kana esculatus)       JO       DevII (37)       Do         Rhinatremid caecilian (Epicrionops marmoratus)       JO       DevII (37)       Do         Amazonian poison dart frog (Dendrobates ventrimacula		JO	RV-tinamou
Örder Squantat (snakes and lizards)         South American pit viper (bohrnops jaranca)       JO       RV-pit viper         Norde American pit viper (bohrnops jaranca)       JO       SpeV (38)         Tuatara (Sphenodon sp.)       JO       SpeV (38)         Order Crocodylia       CT       RV-tuatara         Order Crocodylia       CT       RV-gharialII         Order Chelonia (turtles)       JO       RV-silder turtleI         Red-eared slider turtle (Chrysenys scripta)       JO       RV-silder turtleI         Order Urodela (salamanders and newts)       Palmate newt (Triturus hebveticus)       JO       RV-palmate newtI         Palmate newt (Ambystoma tigrinum)       CT       RV-tiger salamanderII       CT       RV-tiger salamanderIII         Order Gymnophiona (caccilians)       CT       RV-tiger salamanderIII       CT       RV-tiger salamanderIII         Order Anura (frogs and toads)       JO       DevI (37)       JO       DevI (37)         Amazonian poison dart frog (Dendrobates ventrinaculatus)       JO       RV-chinatremid caccilian       DevI (37)         Rocket frog (Colostethus talamancae)       CT       RV-chinatremid caccilian       DevII (37)         Rocket frog (Colostethus talamancae)       JO       RV-derige atlaneated to RV-chinaterenid caccilian       European (Colostethus tala	Pentiles		
South American pit viper (Bohrops jararaca)     JO     RV-Foundo dragon (21)       Order Rhynchocephalia (tutatra)     JO     SpeV (38)       Tuatara (Sphenodon sp.)     JO     RV-Komodo dragon (21)       Order Crecodylia     CT     RV-tuatara       Order Crecodylia     CT     RV-stidler turtle!       Order Crecodylia     JO     RV-stidler turtle!       Order Chelonia (turtles)     JO     RV-stidler turtle!       Red-cared slder turtle (Chrysenys scripta)     JO     RV-stidler turtle!       Order Urodela (salamanders and newts)     JO     RV-stidler turtle!       Palmate newt (Triturus helveticus)     JO     RV-stidler turtle!       Tiger salamander (Ambystoma tigrinum)     JO     RV-tiger salamander!       Order Anura (frogs and toads)     CT     RV-timatremid caecilian       Amazonian poison dart frog (Dendrobates ventrimaculatus)     JO     Devil (37)       JO     RV-tiniatremid caecilian     JO     RV-tiniatremid caecilian       Jo     Revelt (Sana pipiens)     JO     Devil (37)       Amazonian poison dart frog (Dendrobates ventrimaculatus)     JO     Devil (37)       JO     Revlet frog (Colostethus talamancae)     CT     RV-stickleback       Jo     Roket frog (Colostethus talamancae)     CT     RV-stickleback       Order Salmoniormes     J			
Komodo dragon (Varianus komodoenisis)         CT         RV-Komodo dragon (21)           Order Rynchocephalia (tutatan)         JO         SpeV (38)           Tutatara (Sphenodon sp.)         JO         RV-tutatara           Order Crocodylia         CT         RV-tutatara           Order Chelonia (tuttles)         CT         RV-tutatara           Order Chelonia (tuttles)         JO         RV-slider turtleI           Ambidioans         JO         RV-slider turtleI           Amphibians         Order Urdela (salamanders and newts)         Palmate newt (Triturus helveticus)         JO           Palmate newt (Triturus helveticus)         JO         RV-palmate newtI           Tiger salamander (Ambystoma tigrimun)         CT         RV-tiger salamanderII           Order Cymnophiona (caecilians)         CT         RV-thinatremid caecilian           Rhinatremid caecilian (Epicrionops marmoratus)         CT         RV-thinatremid caecilian           Order Anura (frogs and totads)         JO         DevII (37)           Amazonian poison dart frog (Dendrobates ventrimaculatus)         JO         DevII (37)           JO         RV-tiger salamanderII         CT         RV-thinatremid caecilian           Leopard frog (Kana temporaria)         JO         DevIII (37)           Amazonian poison da		JO	RV-pit viper
Order Rhynchocephalia (tuatara)       JO       SpeV (38)         Tuatara (Sphenodon sp.)       CT       RV-tuatara         Order Crocodylia       CT       RV-sharial (Gavialis gangeticus)         Order Crocodylia       CT       RV-sharial (Gavialis gangeticus)         Order Cheonia (turtks)       JO       RV-shider turtleI         Order Crocodylia       JO       RV-shider turtleI         Order Urodela (salamanders and newts)       JO       RV-palmate newtI         Palmate newt ( <i>Triturus helveticus</i> )       JO       RV-palmate newtI         Tiger salamander ( <i>Ambystoma tigrinum</i> )       JO       RV-tiger salamanderII         Order Anura (frogs and toads)       CT       RV-tiger salamanderII         Order Anura (frogs and toads)       JO       DevII (37)         Amazonian poison dart frog (Dendrobates ventrimaculatus)       JO       DevII (37)         JO       RV-tocket frog (Colostelhus talamancae)       CT       RV-tocket frog         Edible frog (Rana excellenta)       JO       RV-teira in frog         Leopard frog (Rana tence)       CT       RV-tocket frog         Leopard frog (Rana excellenta)       JO       RV-trinear deved toad         Leopard frog (Rana excellenta)       JO       RV-tocket frog         Leopard frog (Rana excellenta)<			
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Order Crocodylia     CT     RV-gharialII       Order Chelonia (turtles)     JO     RV-slider turtle1       Ned-ared slider turtle (Chrysemy scripta)     JO     RV-slider turtle1       Join     RV-slider turtle1     JO       Amphibians     JO     RV-palmate newt1       Order Urodela (salamanders and newts)     JO     RV-palmate newt1       Palmate newt (Thurus helveticus)     JO     RV-palmate newt1       Tiger salamander (Ambystoma tigrinum)     JO     RV-tiger salamander1       Order Gymnophiona (caccilians)     CT     RV-tiger salamander1       Rhinaternid caccilian (Criptionops marmoratus)     CT     RV-thinatremid caccilian       Order Anura (fregs and toads)     JO     Devil (37)       Amazonian poison dart frog (Dendrobates ventrimaculatus)     JO     RV-tocket frog       Jo     RV-tocket frog     CT     RV-tocket frog       Edible frog (Rana piens)     JO     RV-tocket frog     Devil (37)       Jo     RV-tocket frog     CT     RV-tocket frog       Ledible frog (Rana piens)     JO     RV-tocket frog       Locard frog (Rana berica)     CT	Tuatara (Sphenodon sp.)	JO	SpeV (38)
Gharial (Gavials gangeticus)     CT     RV-gharialII       Order Chelonia (turtles)     JO     RV-slider turtleI       Red-cared slider turtle (Chrysemys scripta)     JO     RV-slider turtleI       Jona     RV-slider turtleI     JO     RV-slider turtleI       Amphibians     JO     RV-palmate newII     RV-palmate newII       Order Urodela (salamanders and newts)     JO     RV-palmate newII       Tiger salamander (Ambystoma tigrinum)     JO     RV-riger salamanderI       CT     RV-tiger salamanderI     CT     RV-tiger salamanderI       Order Gymnophiona (caccilians)     CT     RV-tiger salamanderII       Order Anura (frogs and toads)     CT     RV-rhinatremid caccilian       Amazonian poison dart frog (Dendrobates ventrimaculatus)     JO     DevII (37)       JO     DevII (37)     JO     DevII (37)       IC     RV-rocket frog     RV-dible frog     DevII (37)       JO     DevII (37)     JO     DevII (37)       IC     RV-dible frog     JO     RV-leopard frog       Ibib frog (Rana esculenta)     JO     RV-leopard frog       Leopard frog (Rana temporaria)     JO     RV-leopard frog       Buropean common frog (Rana temporaria)     JO     RV-brocket frog       Order saterosteiformes     Stickleback (Gasterosteus aculeatus) <td></td> <td>CT</td> <td>RV-tuatara</td>		CT	RV-tuatara
Order Chelonia (uriftes)       Red-eared slider turtle (Chrysemys scripta)       JO       RV-slider turtleI         Need-eared slider turtle (Chrysemys scripta)       JO       RV-slider turtleII         Amphibians       JO       RV-palmate newtI         Order Urodela (salamanders and newts)       JO       RV-palmate newtI         Palmate newt (Titurus helveticus)       JO       RV-palmate newtI         Tiger salamander (Ambystoma tigrinum)       JO       RV-tiger salamanderIII         Order Cymnophiona (caccilians)       CT       RV-triger salamanderIII         Order Anura (frogs and toads)       CT       RV-rhinatremid caccilian         Amazonian poison dart frog (Dendrobates ventrinaculatus)       JO       DevI (37)         JO       DevII (37)       JO       DevII (37)         Amazonian poison dart frog (Dendrobates ventrinaculatus)       JO       DevII (37)         JO       DevII (37)       JO       DevII (37)         Rocket frog (Colostethus talamancae)       CT       RV-rocket frog       Loopard frog         Leopard frog (Rana exilencia)       JO       RV-teorpard frog       RV-teorpard frog         Leopard frog (Rana pipiens)       JO       RV-teorpard frog       Adricen clawed toad (Xenopus laevis)       JO         Pufferian frog (Rana temporaria)       JO		<b>CT</b>	
Red-eared slider turtle (Chrysenys scripta)       JO       RV-slider turtleI         JO       RV-slider turtleI         Jon       RV-slider turtleI         Amphibians       JO       RV-slider turtleI         Order Urodela (salamanders and newts)       JO       RV-palmate newtI         Palmate newt (Triturus helveticus)       JO       RV-palmate newtI         Tiger salamanderI (Ambystoma tigrinum)       JO       RV-tiger salamanderI         Order Gymnophiona (caecilians)       RV-tiger salamanderIII       CT       RV-rhinatremid caecilian         Order Anura (frogs and toads)       CT       RV-rhinatremid caecilian       JO       DevII (37)         Jo       DevII (37)       JO       DevII (37)       JO       DevII (37)         Grade (Colostethus talamancae)       CT       RV-rocket frog       Edible frog       Rua pipiens)       JO       DevII (37)         Rocket frog (Colostethus talamancae)       CT       RV-rocket frog       Edible frog       Rua pipiens)       JO       RV-sticker frog         Leopard frog (Rana pipiens)       JO       RV-tricen clawed toad       RV-stickel back       RV-stickel back         Order Gasterostetiformes       JO       RV-stickelback       Grader sciekelback       Grader sciekelback       Grader sciekelback <td< td=""><td></td><td>CI</td><td>RV-gharialII</td></td<>		CI	RV-gharialII
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Amphibians         Order Urodela (salamanders and newts)         Palmate newt ( <i>Triturus helveticus</i> )       JO       RV-palmate newtI         Tiger salamander ( <i>Ambystoma tigrinum</i> )       JO       RV-tiger salamanderI         Tiger salamander ( <i>Ambystoma tigrinum</i> )       JO       RV-tiger salamanderII         Order Gymnophiona (caecilians)       CT       RV-tiger salamanderIII         Order Anura (frogs and toads)       CT       RV-rhinatremid caecilian         Amazonian poison dart frog ( <i>Dendrobates ventrimaculatus</i> )       JO       DevII (37)         JO       DevIII (37)       JO       DevIII (37)         Rocket frog ( <i>Colostethus talamancae</i> )       CT       RV-rocket frog       Edible frog         Edible frog ( <i>Rana pipiens</i> )       JO       RV-elible frog       RV-leopard frog         Leopard frog ( <i>Rana pipiens</i> )       JO       RV-European frog       RV-leopard frog         Painted frog ( <i>Discoglossus galganoi</i> )       JO       RV-European frog       RV-painted frog         Stickleback ( <i>Gasterostetins aculatus</i> )       JO       RV-stickleback       Corder Sumoniformes         Stickleback ( <i>Gasterosteting aculatus</i> )       JO       RV-brook trout       Brown trout ( <i>Salveinus fontinalis</i> )       CT       RV-brook trout         Brown trout ( <i>Salweinus fontinalis</i> )       CT	Red-eared sider turtle (Chryseniys scripta)		
Order Urodela (salamanders and newts)       JO       RV-palmate newt I         Palmate newt ( <i>Triturus helveticus</i> )       JO       RV-palmate newtII         Tiger salamander ( <i>Ambystoma tigrinum</i> )       JO       RV-tiger salamanderI         Tiger salamander ( <i>Ambystoma tigrinum</i> )       G       RV-tiger salamanderII         Order Gymnophiona (caccilians)       RV-tiger salamanderII       RV-tiger salamanderIII         Order Anura (frogs and toads)       CT       RV-thinatremid caccilian         Amazonian poison dart frog ( <i>Dendrobates ventrimaculatus</i> )       JO       DevI (37)         JO       DevII (37)       DevII (37)         Rocket frog ( <i>Colostehus talamancae</i> )       CT       RV-roket frog         Leopard frog ( <i>Rana ippiens</i> )       JO       RV-teopard frog         Leopard frog ( <i>Rana ippiens</i> )       JO       RV-teopard frog         European common frog ( <i>Rana temporaria</i> )       JO       RV-berian frog         African clawed toad ( <i>Xenopus laevis</i> )       JO       RV-brow trout         Painted frog ( <i>Corogouss galganoi</i> )       CT       RV-brow trout         Order Salterosteus       G       RV-painte frog         Stickleback ( <i>Gasterosteus aculatus</i> )       JO       RV-brown trout         Painted frog ( <i>Discoglossus galganoi</i> )       CT       RV-brown trout	Amphibianc		
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Order Lamniformes			
Order Lamniformes	Partiloginous fich		
		СТ	RV-lemon shark (21)

<sup>a</sup> References are provided for previously characterized viruses.

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SFVL3	FRPOROYPINPKAKASIOTVINDLLKQGVLIQONSIMNTPVYPVFKPDGKWRMVLDYREVNKTIPLIAAQNQHSAGILST-IFRGKYKTTL
HSV	PRPOROYPINPKAKASIQIVIDDLLKQGVITPQNSTMNTPVYPVPKPDGRWRMVLDYREVNKTIPLTAAQNQHSAGILAT-IVRQKYKTTL
SFV1	PRPOKOVPINPKAKPSIQIVIDDLLKOGVLIQQNSTMNTPVYPVPKPDGKWRMVLDYREVNKTIPLIAAQNQHSAGILST-IYRGKYKTTL
HERV.L	IVIQKHYHHPGG?IAEISATIKDLKNAGVVIPITSLFNSPFWPVQKTDGSWRMRVDYHKLNQVVTPIAAAIQDVVSLLKQITSPGTLYAAI
SpeV	PPPQMQYKYPAETEKGIQAMIDSLLRQGVVVKMQSVCNSPIWPVIKADEG?DLPDDCRLL?????PFAPVVAKYNEIVAA-IPWGPAGTVI
SnRV	PKMIKQYPVPDASHASIKETVEALLEQGVLRKCNSTVNSAIWPVGKPDGSWRLTIDYRPLNSAVSCPYPTVASTPELFAKLEKKYQVYSSL
<b>RV</b> Painted Frog	PQWQKQYPVPKESVASITEMIEVLIKSGNGYACYSPCWPVRKAVLKGNED-EKAASSWRLLIDYRAVNKLAKRMAPVVFVYCEMMSKISPEMKVFSVI
RV Tinamou	$vtnvpq hpisaaarngiaeviadlgke?misr-scspc*stvwpvrkpdgr+wrltidyrrlnantvpltaavpnianltatlaaa\texttt{hpwratlabarpwr$
<b>RV</b> Common Possum	IITLKQYHVPGGQ?NBIANTIKEYVEAGVLVP??SPYN?PVWLVQKSDGTWRMTVDYRQLNKVTPPLYAIVPDTVTLIERIQKYDGWYTVI
	IVVHTEQFK?TRCPTQALEETIHDLREVGVVPVKSPYNSPVMPVRKPDSWRLTVDYRKLNAATPPLHAAVPSMATLVQAIETLGTWYAVV
HERVJ	VVRRQFPPLESMLGLKPIIESLINDGLLEPCMSPYNTPLLPVKKSDGSYELVKDLRAINQTVQTTNPVVPNPVTLLSKIPVNHQWFTVI
RV Sparrow BV Lamon Shork	PAQDKQYPISPEGQRGLTAVIQGLMEEGILEPCMSPQNTPMLAVKKADGTYELVQDLGEINK??ISR*PIVSNPYPLLSQIPQEHAWFSVI EVRVRQYPISWEGQQGLKDVMRDLITDGLVEPCMSRYNSPILPVRKSDGSYRMVQDLREINKIVQIRHPVVPNPYTILGRIPPDHGWFSVV
RV Lemon Shark RV Rock Wallaby	zvrvqifiswbayqdalavdyrddi ibdivefcabringfiafvrabdsYRWyQDLREINRIVQIAREVYFRFIIBGRFFDABWFSVI VVRIKEYPVSLBGRG7KPGIEELLATGLIET-*SPFNIPIFPV7KKDGKYRWYQDLREINRIVUSSYFVLPPPYTILGRIFEDBTWFSVI
RV Komodo Dragon	VVRQR0YPILMEGRGLRPVEGLIEDGLEPCMSPFNTSILPIRKADGTYCLIQDLQRVNEIVJVENPYTLSFIPHSKIPHSVA
RV S.Faced Dunnartl I	FPNKRQYPIKQEDRIGLQPLIDKFLKYGLLAPCHSPCNTPILPVKKPNGEYRLVQDLRAINDAVIPIHPIVPNAYTIFAQIPGNTQWFSTL
ERV-9	FPYQRQYPLRPEAHKGLQNIVKHLKAQGLVRKCSSPCNTPILEVQR???????PLVQDLRLINEAVISLYFVVPNPYTLLSQIPEEAEWFTVL
GaLV	PVAVRQYPMSKEAREGIRPHIQKFLDLGVLVPCRSPWNTPLLPVKKPGTNDYRPVQDLREINKRVQDIHPTLPNPYNLLSSLPPSYTWYSVL
FeLV	PISIRQYPMPHEAYQGIKPHIRRMLDQGILKPCQSPWNTPLLPVKKPGTKDYRPVQDLREVNKRVEDIHPTVPNPYNLLSTLPPSHPWYTVL
HERV.E	PVRQKQYPVLREALEGIQVHLKCLRTFRIIVPCQSPWNTPLLPVPKPGTKYRPVQDLRLVNQATVTLHPTVPNLYTLLGLLPAEDSWFTCL
BoEV	PVRKRQYPMPTEARIGILPHISRLKRTGILVECQSAWNTPILPVKKEGGQCRPVQDLR*VNQATVTLHPTVPNPYTLLSLLPPKTRIFTCL
DevI	YPRLPRYPLKAAQBQSLGVQITSLLRSGVLIRCVSPCNTPLFPVKKKTAPGDPPKYRLVQDLRAVNSATILETPVVPNPNTLLSQVPTSATLFTVI
DevII	eq:plulkgyplclakstamsrdikenvkagvlvQrsppcmtplypikekkgpkgsphrmahdlraindvtesvtsifpnphtvfsQiavmskcftiting and a statemeter and a sta
DevIII	YPRKAQARPR???SQAASDQLKVYLEIGIIVPRTSPCNTLLFPVKKKTVKGEPAKVRMVHDLRAVNDVMEFETPIVFNPHTLLSNVPATAKVFTVT
<b>RV</b> African Clawed Toad	eq:lprpqyplkpdqsaaitkqlqslldngaikrqsspcntplppvkkkqgg?pdkyrlvqdlravnea?vmetplvsnphtllsglppaatyftaiterterseterseterseterseterseterseterset
RV Rocket Frog	LPRIPQYPLKAIQEDSLKAQIDALLTQGALVPCISECNTPLPPVQKKVPKGQPPKFRLVQDLRALNAATILETPVVPNPHTLLSAIPPEAAYFTVV
RV Pufferfish	???????REIGIDGTTPDIG??LPRNISVLS%???????RKPSNGWRIVRDUQAVNAAVIREHEIVPDPSTLINDIRSDKLFSVI
RV Freshwater Houting RV Stickleback	RPYKAHYPLSKEAEKGIIPIHSALDERGALVP-CPNSPCNTPILPVRKPSGDYRFVQDLRIVNDAVHARARLVPNTVTILSQVPPGSC?FSVI RPHKHQYPLRQEAIDGITPVFNSS*RPGVIVP-CPDSPVRTPIFPVKKIDAGKPTGWRFVQDLKAVNAAVHARAPNVPNPYTIMAQVPPDARWFSVV
RV Gharial	RPHRNQTELWQATIGTTEVINSTWEGTVET-CEDSTWITTEFTVALIDAGT-ACTIONRTVQULAAVMAAMARAEWVENETTIBAQUFEDARWESVV PPCVPQYELWKEAEEGLQELIDESTRIKGILVET-COSPERTYPVLPVRKFRKRGKDGKEVYRVQULAAVMAAVVPNPATTUTSTEWWARTRFTVI
RV Pit Viper	PLCTPQ/FLMNARESJQFL/IDE/INFX/IQU/LCKCTSLCNKP/FPVRKPKLD-QDRNPVFRFV?VLR/NQSONFCCPDVPNRATIITEVPASATYFTVI
RV Slider TurtleII	PSIPOYPLSLEAREGIRFIIEGFIAOKUVRP-ORPCNTPILPVKKLPKK-EGPVKWRFVODLCLINRVVIPLHAVVPDPATIISOIPWDAKWFTVI
RV Slider TurtleI	LPRVSQY?LSKQAEBGIHPVLTSLIEQGVSVPTTSOCNTPILPVRKPGKQSWHFVQDLRAINAPVVPTFPVVPNPATILSCIPPSATVFTVV
RV Tuatura	LPRISOYPLRPEAEEGILPVIKSLLDOGILVPNRSOCNTPILPVKKPGKNVYRFVODLRSINSVVPPTFPVVPNPATILACIPSTAKYFTAV
<b>RV</b> Palmate NewtI	LPRIRQYRLPPEAVAALSKIIDELMAQGVVVE-TDSNVCNSPIIPVMKRTDPNAPPVYRLVIDLRELNKCIIPQYPVVPDISSLLTAIDSEAMFFTVI
RV Palmate NewtII	LPRIPQY?ISRDGERALLQTIQDLIRSGVVEE-TDSNSCNSPILPILKRPSSSKGTPDVYRFVIDLRHNNKVVEPMYPVVSDITTILTMIPPSAKYFSVL
<b>RV Edible Frog</b>	PHFQKPYPLAEEKIQGITPLIEEYKKEGILKEIVTPWNTPINPVRKANGQWRLVQDLRGINRVIIQFPPLVADITSVFGSISSDSCVYTVI
RV European Frog	PIFQKQYPLSEEKLNGIDPQID?YLQKGILTPVISPWNTPINPVRKANGTWRLVQDLRPLNKCITPLPPLVADISTIFAPLTPEHKWYTVI
<b>RV</b> Leopard Frog	PFFQKQYHLHMVKVQGIEPQITTYIQ?GILKTVISPW?THINPVRKANGEWRLVQDLRGVNKCI*PLPPLVSDITGIFVPASSKMYMVL
	GPKLSQYPLHPEAVKGVR?VNESLLLDTDYRPSMSPYNMSLFPVKKSS*KWHIAHDLRALNEKVQAEFPCVENPATVLQQ-TPLGGAHTVL
RV Tiger SalamanderII	LP*VKQYPVLKEVDEAIYETFATLVRQGISVPSESPCNMVVYSIEKAKGGSWRFILDACP????????PNSATMLCNIPLRNQYFIVI
RV Tiger Salamander111	LP*ARQYPMSREVDEGIDYISL??MEQGI?????QSPCNLAVYPVKKSQGEAWRLIQDMRAIYQIVEAEPHLIPNPGTLCSNSTEP*NFTAI
WDSV RV Iberian Frog	LPSIRQYPLPKDKTEGLRPLISSLENQGILIKCHSPCNTPIFPIKKAGRDEYRMIHDLRAINNIVAPLTAVVASPTTVLSN-LAPSLWFTVI VPRLPQYPLSPDSVEGIRPVIEELLQEGVLTPCRSQANTPIYPVPKPIKQ-GEDRCRWRLVHDLRAINKVIIPSVPVVPNPSTILATIPPTATHFTVI
RV Horse	VPRLPQTELSEDSVBJTREVIDELEDDEGUTTFCRSQAMTFTTFVTRTRQ-GEDRARMKLVNDLRAINKVITFSVFVVPRFTILATIFTVT CPSVAOHPLSLDPNTGKR7????LLQOVLLF-CTSSCDAPTFPVREGKFSSSGNQIYRVODLRAINKVITFSVFVPNEVPIRAVILASTPADAAWFTGT
H-Cosmid(U85A3)	LSNLKHYPLÓKEAIGGIVPVIQVLKNGLIP-CTIP*NSPIPVKKLSGRGWKPVQELAINNIIILHHTVPNPQTLLSPISTFSYFSVV
EIAV	GEKIPOWPLTKEKLEGAKEIVORLLSEGKISEASDNNPYNSPIFVIKKKSGKWELLODLRELNKTVOVGTEISEGLPHEGGLIKCKHMYVL
OMVV	GPHIAOWPLTOBKLEGLKEIVDKL??EGKVGRAPPHWTCNTPIFCIKKKSGKWRMLIDFRELNKOTEDLAEAOLGLPHPGGLOKKKHVTIL
SIVmac	GPKLKQWPLSKEKIVALREICEKM??DGQLEEAPPTNPYNTPTFAIKKKDKN?WRMLIDFRELNRVTQDFTEVQLGIPHPAGLAKRKRITVL
HTLVI	APRNQPVPFKPERLQALQHLVRKALEAGHIEPYTGPGNNPVFPVKKANGTWRFIHDLRATNSLTIDLSSSSPGPPDLSSL-PTTLAHLQTI
BLV	PT*GASIPFKLERLQALQDLVHRSLEAGYISPWDGPGNNPVFPVRKPNGAWRFVHDLRATNALTKPIPALSPGPPDLTAI-PTHPPHIICL
RSV	PVWIDQWPLPEGKLVALTQLVEKELQLGHIEPSLSCWNTPVFVIRKASGSYRLLHDLRAVNAKLVPFGAVQQGAPVLSALPRGWPLMVL
LDV	PVWIEQWPLTAQKLDAVQNIIQDLLKDGRIIPSRSQWNSPIFVIQKKDKSKFRMLHDLRAVNALIKDWGALQPGTPWPGAIPSEWPVIAM
HERV K10	PVWVEQWPLPKEKLGVLYKI???LLKKGYISP-TFSPWNSPVFVIKKKSGRWRQRCN??GVNAVIQPMGALQPGLPSPTVLPKD*PLVII
IAP Hamster	PLWVSQWPLSSEKLEAVTRLVQEQERLGHLEPSTSPWNTPIFVIKKKSGKWRLLHDLRAINNQMHLFGPVQRGLPLLSALPQDWKLIII
RV Bower Bird	PIWIDOWPLSKEKLAHVERLVEDOLRRGHVIPSTSPWNTPIFAIPKKSCKWRLHDLRAINSVMQEMGHLORGLPSQSMLPVNWQLLVV
RV S.Faced DunnartI SRV1	PIWDOWFLSNEKITTLKSIIMEQQEKLGHEH-SFSAYNSPVFVIRKEQ?WRML?DLRAVNASMQPMGALQPGLLSPNMVPKEYHMVII
Jaagsiekte	PVWVDQWPLTSEKLAAAQQLVQEQLEAGHITESNSPWNTPIFVIKKKSGKWRLLQDLRAVNATMVLMGALQPGLPSPVAIPQGYLKIII PVWVDQWPLTQEKLSAAQQLVQEQLRLGHIEPSTSAWNSPIFVIKKKSGKWRLLQDLRKVNETMMHMGALQPGLPTPSAIPDKSYIIVI
MMTV	eventowersteansaaduvvoolatigatie==sispennitpvevikkksk=======kaligolakivneinnakalopgibespa=+poksiiivi Pvelioneelkoekloolotolotaliee==sispennitpvevikkksk======kaligolakivnatmenokalopgibespa=+poksiiivi

FIG. 2. Amino acid alignment derived from retroviral reverse transcriptase proteins, based on that described by Xiong and Eickbush (45). Sequences identified in this study are indicated in bold type. The underlined regions of the alignment were used in subsequent phylogenetic analysis. Asterisks represent in-frame stop codons, and missing data (due to frameshifts or deletions) are indicated by question marks. Three isolates, RV-brook trout, RV-brown trout, and RV-tiger salamanderI, contained large deletions and are not shown.

other, and only in those cases where homology was less than 90% at the nucleotide level were both clones investigated further. Thirty novel endogenous retroviral fragments were characterized by sequencing the entire length of the amplified and cloned fragment. Of these, 3 were derived from nonplacental mammals (marsupials and monotremes), 2 were from birds, 5 were from reptiles, 14 were from amphibians, and 5 were from bony fish (Table 2). The retroviral isolates included representatives from each of the four orders of reptiles and the three orders of amphibians. Southern hybridization (32) of each of the fragments was performed to confirm its species of origin (data not shown). The lack of sufficient genomic DNA meant that it was not possible to accurately determine the copy number of many of the isolates.

Conceptual translations of the DNA sequence derived from

each isolate were performed, and a 160-amino-acid-residue region of the reverse transcriptase protein was then aligned to that of other previously described retroviruses, as described by Xiong and Eickbush (45). Many of the sequences contained stop codons and frameshifts, which required the insertion of one to several nucleotides (coded as unknown) in order to maintain the reading frame (Fig. 2). This alignment was used as the basis for the phylogenetic analyses. The majority of sequence information from outside of this region was not suitable for phylogenetic reconstruction due to the difficulty of aligning homologous amino acid positions, although it was possible to include a 30-amino-acid-residue region from the protease protein. Both neighbor-joining and maximum parsimony trees were generated from the alignment, with support for individual branches investigated by bootstrap analysis.

	101	???
SFVL3		-GQQYCWTRLPQGFLNSPALFTADVVDLLKEVPNVOVYVDD
HSV		-GKOYCWTRLPOGFLNSPALFTADVVDLLKEIPNVOVYVDD
SFV1		-GKQYCWTRLPQGFLNSPALFTADVVDLLKEIPNVOAYVDD
HERV.L		-GQQCIFTVLPQGYINSPALCHNLIQSDLDHFSLPQDITLVHYIDD
SpeV		-NQQYSFTRTPQGFHSSPSICHSVVSKMWDKLKPESRGCVLSYVDD
SnRV	DISNGFWSIRLEEECQYLFAFTFD	-TQQYTWTRLPQGFHASPGIFHQALYNGLASCKTAIESQ-GCKLLQYVDD
<b>RV</b> Painted Frog	DVANGFWSIPLAEECQYRTAFTWV	-GRQYAFTVLPQGYCDSSAIFHTYMKNALTSFPTSR?DRIIQYVDD
RV Tinamou	DVKDVFFMAPLQEKDREKFTFTWD	-GIQYTFNGLPQGYKHSPTIAHAALAGLLQKVSLPREVKLYQYVDD
<b>RV</b> Common Possum		-GRQYIFTRLPQGDLHSPTICHRIVAEHLDKLKLAGIQLTHYVDD
		-GRQYTFTRLPQGYIHSPTICHEQVALTLEKIKERLPASVKLHHYVDD
HERV.I		-*KQYQYTVLPQGFMDSPNLFGQILEQVLKDVSVPKQLCLLQYVDD
RV Sparrow		-KQQHRWTCLPQGFTESPNLFGRALEDPLKSSVPGEEIQILQYVDD
RV Lemon Shark		-KQQYRWTVLPQGFTESPNLFGQILERVLEELVLPPILKFIQYVDD
RV Rock Wallaby		-RQQYRWCVLPQGYTESPNLFGQVLEGILEGFDPMARTRVLQYVDD
RV Komodo Dragon RV Stripe Faced DunnartII		-RQQLRWTTLPQGFTESPNLFGQALEQLLEKFRVPEGTALLQYVDD
ERV-9		-PYQLTWLVLPQGFRDSPHLFGQALAKDLRDLELKGSCLLQYVDD
GaLV		-TSQLTWMVLPQGFRDSPHLFGQALAKDLGHFSSPGTLVLQYVDD -TGQLTWTRLPQGFKNSPTLFDEALHRDLAPFRALHPQVVLLQYVDD
FeLV		-SQGLTWTRLPQGFKNSPTLFDEALHSDLADFRVRYPQVVLLQYVDD -SQGLTWTRLPQGFKNSPTLFDEALHSDLADFRVRYPALVLLQYVDD
HERV.E		-TTQYTWTQLPQRFKNSPTIFGEALARDLQKFPTRDLGCVLLQYVDD
BoEV		-TIQITWIQLPQRFRNSPTIFGEALARDLQRFFTRDLGCVLLQYVDD -KQQLTWTRFPQGFKNAPSIFGEALASDLEPFQPERYGCWLLQYVDD
DevI		-GSQLTWTRTPQGFRNAPSIFGEALASDLEFFQFERIGCWLLQIVDD -GSQLTWTRTPQGAQNSPNQFTHAMKMTLDPWVLQNLHVTLLQYVDD
DevII		-GSQLIWIRIPQGAQNSPNQFTHAMKMILDPWVLQNL,HVILLQYVDD -GKQY*WTVLPQGGKNSPTIYSTALAGILEQWQPPHSQIVLLQYVDD
DevIII		-R?QHTWTVMPQRAQNSPSQFT????????NWVT*HAETLLQYVDD
RV African Clawed Toad		-RQQYVWFVLPQGAQNSPTHFSRALTSILDSWISTHPEITLLQYVDD
RV Rocket Frog		-GRQLTWTVMPQGAQNSPNQFTQAMQQCLQPWKDANPGITLLQYVDD
RV Pufferfish		-GK*YTWTRLPQGYCESPTIFSQVMSPCMSKFNTPKGSQILLYVDD
<b>RV</b> Freshwater Houting		??????????????????????????????????????
RV Stickleback		-GKPYTFTRLCQGFTESPTIYNEALRESLESLTLSPGTALLOYVED
RV Gharial		-KQHLTWTRLPQGYTKSPTIFSQILRRDLQGIILTLDTVLIQYVDD
RV Pit Viper		-DQQYTWTCLSQEYTESPHPFLQILKTDLSSIALPGGSTLIQYVDD
<b>RV</b> Slider TurtleII	DLKSAFFSIPVHPDSQYLFGFTWE	-GQSYVWQRLPQGFQDSPTIFSQCLRHDLEGFACSEGSTLVLYVDD
<b>RV Slider TurtleI</b>	DLCSAFFSIPIHPDSQYLFAFSFL	-GLQYTWTRLPQGYTESPTLFSQILRWDLADLIFPMGSTLV?YVDD
RV Tuatura	DLCSAFFSIPIHCDSQYLFAFTYR	-GCQYTWTRLPQGYVESPSLFSQILKKDLDDVRFQEGSVLVQYVDD
<b>RV</b> Palmate NewtI		-GRSYMFTRAPQGFTESPSIYSQALKRQLDNIHMPTDTALLQYVDD
RV Palmate NewtII		-GRSFRFCRIPQGYTESPSIYSQALKAQLDDLELSQGNVLVQYMDD
RV Edible Frog		-PGQLTWTGLAQGFVDSPAAFSMVLKRSLDDWTPSQGSILLQYVDD
RV European Frog		-RGQLTWTRLPQGFVDSPAAFSMVLNQSLTDWVPEHGSVLIQYVDD
RV Leopard Frog		-LGQLAWARLPQGFVDHPTAFSMVLKVTLDDWGPPQGSVLLQYVDD
		-GQSYQWTQLPQGYTESPSVFSRVLAHDLADFKSCLPAHVKLIQYVDD
RV Tiger SalamanderII		-RIRLQHSRLPQGYCVSPSVFNKVFHTYLGNIQTDSTIIQYVDD
RV Tiger SalamanderIII WDSV		-QTKLKSTCLPQGYYESPLVFNRVLQMGVGNITMENSVVLQYVDD
RV Iberian Frog		-GHQYTWTVLPQGFIHSPTLFSQALYQSLHKIKFKISSEICIYMDD
RV Horse		-KRQLTWTRLPMGYHDSPSCFSQALKDKLDEWQPSGDSILVQYVDD -GRQFTRTPIPQGYCESPSIFSOVLKADLDQPSAAF?FKOGSTPVOYVDD
H-Cosmid(U85A3)		-EWQYTWTVMPQRYTESPT*CSQILEADLE??????????????????????????????????
EIAV		PDKRYVWNCLPQGFVLSPYIYQKTLQEILQPFRERYPEVQLYQYMDD
OMVV		PCTRYYWKVLPQGWKLSPSVYQFTMQEILRDWIAkHPMIQFGIYMDD
SIVmac		PGKRYIYKVLPQGWKGSPAIFQYTMRHVLEPFRKANPDVTLVQYMDD
HTLVI		PGTRYAWRVLPQGFKNSPTLFEMOLAHILOPIROAFPOCTILOYMDD
BLV		PHRRFAWRVLPQGFINSPALFERALQEPLRQVSAAFSQSLLVSYMDD
RSV		PARRFQWKVLPQGMTCSPTICQLVVGQVLEPLRLkHPSLCMLHYMDD
LDV		PAKRYQWTVLPQGMKNSPYICQQVVAEVIRPIRERFRDAVIIHYMDD
HERV K10		PAAKYHWKVLPQGMLNSPTVCQTFVGRTIQPVRDQFPDL????????
IAP Hamster		PDKRFQWKVLPQGMANSPTICQLYVQEALEPIRKQFTSLIVIHYMDD
RV Bower Bird		PTKRYQWVVLPQGMHNSPIMCQLYVAWALAPLRKQYPQYLIYHYMDD
<b>RV</b> Stripe Faced DunnartI		PAPRWQWKVLPQEMANSPILCQ??VDKVLAPVRNLYPNVYMLHYMDD
SRV1	DLKDCFFSIPLHPSDQKRFAFSLPSTNFKE	PMQRFQWKVLPQRMANSPTLCQKYVATAIHKVRHAWKQMYIIHYMDD
Jaagsiekte	DLKDCFYTIPLAPQDCKRFAFSLPSVNFKE	PMQRYQWRVLPQGMTNSPTLCQKFVATAIAPVRQRFPQLYLVHYMDD
MMTV	DLQDCFFNIKLHPEDCKRFAFSVPSPNFKR	PYQRFQWKVLPQGMKNSPTLCQKFVDKAILTVRDkYQDSYIVHYMDD

FIG. 2-Continued.

Figure 3a shows an unrooted, bootstrapped neighbor-joining tree of the isolates shown in Fig. 2 (groups not recovered in at least 50% of the bootstrap replicates were collapsed and are represented by polytomies). It was apparent from this analysis that the retroviral sequences clustered into three main groups, each with bootstrap support greater than 80%.

One group (group I) contained the spumaviruses (human spumavirus [HSV], simian foamy virus type 1 [SFV1], SFVL3, and FeSFV), the previously described *Sphenodon* endogenous retrovirus (SpeV, isolated from a reptile), snakehead fish retrovirus (SnRV), HERV.L (a recently identified endogenous human retrovirus), and MuERV.L (an endogenous murine retrovirus closely related to HERV.L), as well as several other novel sequences derived from marsupials, birds, and amphibians. In contrast, group II contained viruses derived exclusively from mammalian and avian hosts and included all members of five of the seven currently recognized retroviral genera (the lentiviruses, the HTLV-bovine leukemia virus [BLV] group,

the ALVs, and the type B and D retroviruses), as well as several novel endogenous viruses. Investigation of 30 other amphibian, reptilian, and piscine hosts with the EM primer failed to reveal retroviral sequences related to this group. Group III contained the majority of the endogenous retroviral sequences isolated by our PCR screening, as well as all members of the murine leukemia virus (MLV) genus, exemplified by the feline leukemia virus (FeLV), gibbon ape leukemia virus (GaLV), human endogenous retrovirus type E (HERV.E), and bovine endogenous virus (BoEV). Members of the MLV genus have previously been identified in several species of birds and reptiles (4, 19, 29, 35, 42, 48) and, consistent with this, we also identified elements clustering with the FeLV/GaLV/BoEV/ HERV.E isolates in both of these vertebrate classes (data not shown). Several other previously described viruses were also present within this lineage. These included the human endogenous retroviruses ERV-9 and HERV.I (44), the recently described walleye dermal sarcoma virus (WDSV) from fish, and



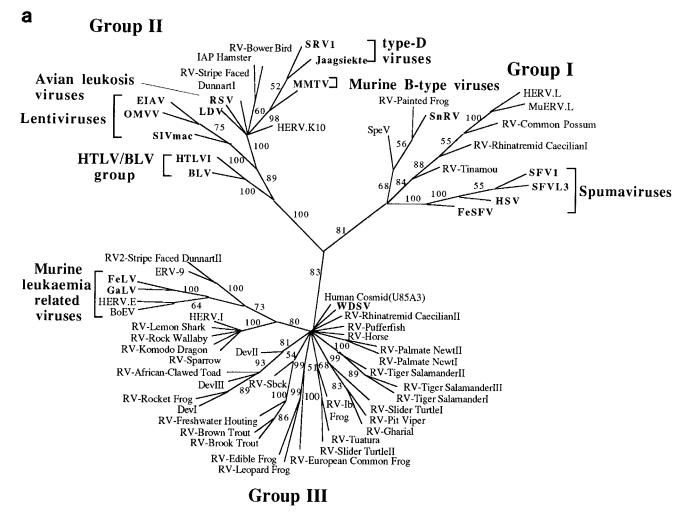


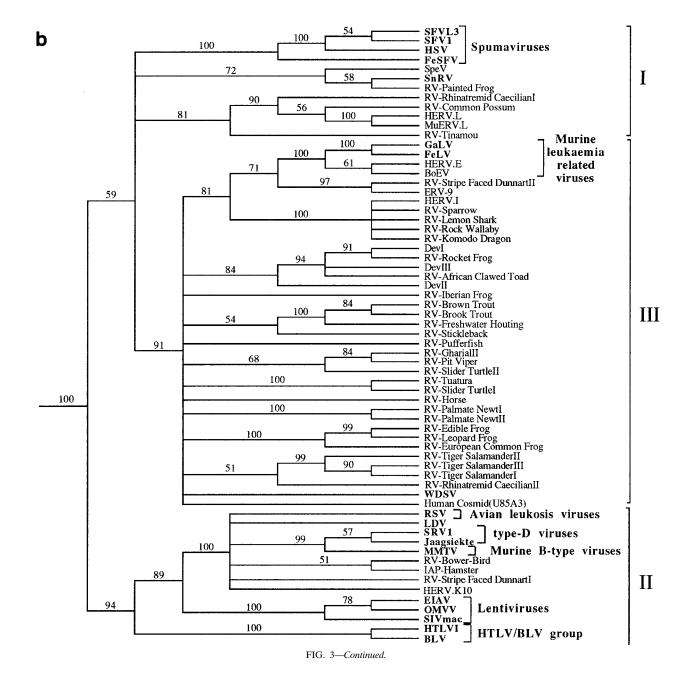
FIG. 3. Phylogenetic trees of the retroviruses based on the alignment shown in Fig. 2, with the addition of 30 residues derived from the protease protein. Exogenous isolates are indicated by bold type. The genera to which particular retroviruses have been assigned are also shown. (a) Unrooted neighbor-joining bootstrap tree. The figures on each branch represent bootstrap support (from 1,000 replicates), with percentage values on only those branches with support greater than 50 being shown. Unresolved branches are shown as polytomies. (b) Neighbor-joining tree rooted on several *gypsy* LTR retrotransposon sequences (*gypsy*, *Del*, *Ty3*, and *micropia*), with figures on each branch again showing percentage bootstrap support (1,000 replicates). (c) Rooted neighbor-joining tree with branch lengths proportional to the genetic distance between the sequences. The tree is rooted as described in the legend to panel a. (d) Strict consensus of six equal-length trees identified by maximum parsimony. Bootstrap values were generated from 100 replicates (each of 25 random-sequence additions) by using the unordered matrix. The tree is rooted as described in the legend to panel a.

the *Dendrobates* elements (DevI, DevII, and DevIII) from a poison dart frog. A BLAST search revealed that one further retroviral sequence should also be included. This element was present in a cosmid (accession no. U85A3) sequenced as part of the human genome mapping project (25). One further point to note is that the entire nucleotide sequence of the WDSV isolate has already been determined and contains at least two novel accessory genes (15). This, and other evidence, has led to the suggestion that it probably represents a novel retroviral subfamily or genus (15, 30).

Several gypsy LTR retrotransposon sequences (gypsy, del, *Ty3*, and *micropia*) were added to the alignment and used as outgroups to root the retrovirus tree. In rooted phylogenetic analyses, the group II sequences were recovered in a well-supported clade that is the sister group to all other retroviruses (Fig. 3b). Group III sequences also comprised a well-supported clade. However, the addition of the outgroup sequences abolished the bootstrap support for the group I spumaviruslike sequences shown in the unrooted analyses, breaking them up

into three well-supported clades that form a polytomy with the group III sequences. Support for this basal polytomy (59%) is also uncompelling. Varying the composition of the outgroup sometimes resulted in weak bootstrap support (about 60%) for the monophyly of group I or an association of the spumaviruses with the SpeV, SnRV, RV-painted frog subgroup. Individual rooted neighbor-joining and parsimony trees did usually include the group I sequences as a clade (Fig. 3c and d), but this monophyly was also dependent on the composition of the group I spumaviruslike sequences close to the base of the retrovirus tree and the weak support for the clade comprising group I and group III sequences must also limit confidence in the placement of the root of the *Retroviridae*.

The complex and diverse nature of retroviruses clustering with the MLV genus is emphasized in Fig. 3b. Most of the relationships within this lineage were not well supported by bootstrap analysis, and the reason for this lack of resolution is apparent from Fig. 3c and d: the distance from the base of the



group to the separation points of many of the taxa is small when compared to their overall diversity. Despite this, it did appear that there were several well-supported subgroups which contained members derived from only a single vertebrate class or order. For example, many of the fish isolates clustered together and groups of amphibian viruses were also observed. To determine whether this clustering was statistically significant and to try and increase the resolution of the various taxa, the group III viruses were then analyzed separately from the other retroviral sequences. This enabled trees to be generated from an extended sequence alignment (257 as opposed to 190 amino acids), as shown in Fig. 4. The degree to which viruses clustered on the basis of their host class of origin was assessed using the program MacClade (20). The number of steps required to generate the actual host class-virus associations shown in Fig. 4 (nine) was always significantly lower than that observed for each of 100 test replicates in which the host class characters were shuffled randomly between the viral taxa (range, 18 to 24 steps; P < 0.01).

# DISCUSSION

Retroviral phylogeny and taxonomy has for a long time been based almost exclusively on viruses within mammalian and avian hosts, and with the exception of several MLV-related retroviruses within reptiles (for which no sequence data have been reported), all members of the seven currently recognized retroviral genera are present within one or both of these vertebrate classes (4, 9, 19, 48). We, and others, have previously obtained molecular data from a small number of reptilian,

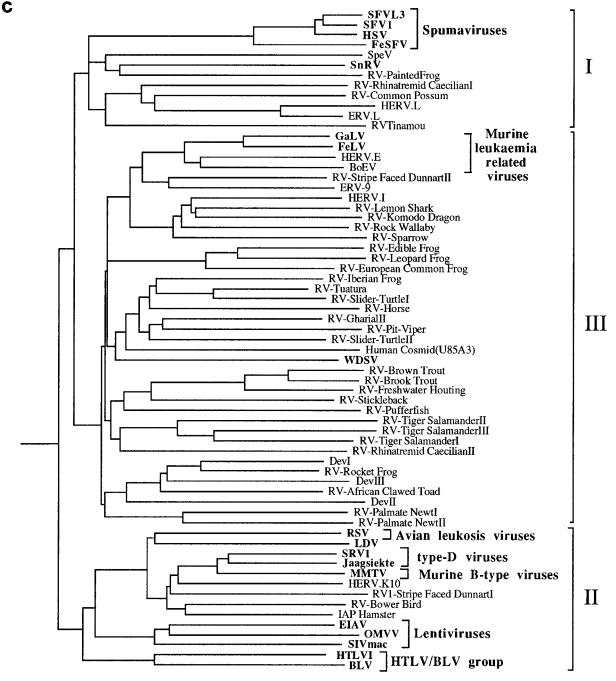
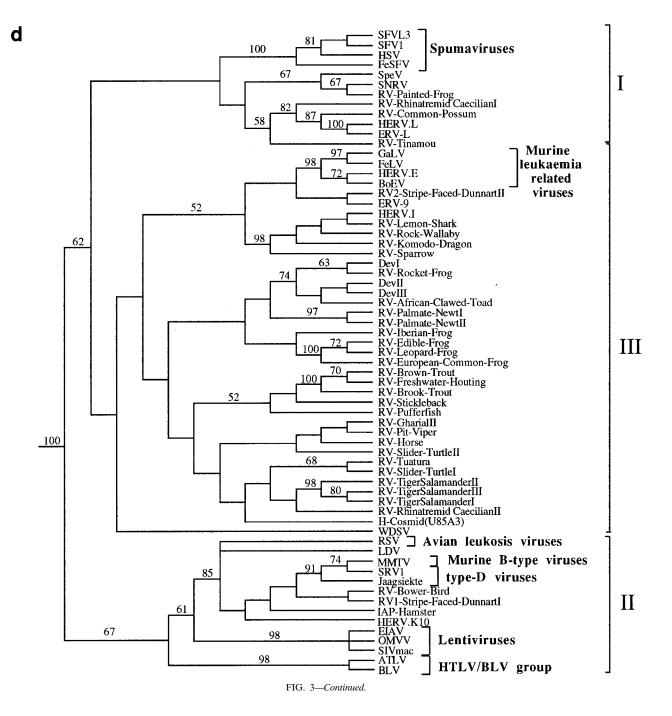


FIG. 3-Continued.

amphibian, and piscine retroviruses (13, 15, 22, 38, 39); those sequences, together with the ones in this report, create an emerging picture of the distribution and diversity of retroviruses within lower vertebrate taxa. Endogenous retroviral sequences have now been identified in more than 25 vertebrate orders across six classes, and this suggests that retroviruses may well be ubiquitous in many vertebrate taxa, although their exact host range remains to be determined. The most basal vertebrate from which a retroviral sequence has so far been identified is the lemon shark *Negaprion brevirostris* (22). We think it unlikely that this element represents a particularly primitive or basal virus for two reasons. First, it is closely related to viruses within several different vertebrate classes, including HERV.I within humans. Second, rooted topological constraint trees which placed RV-lemon shark (or all of the HERV.I-related retroviruses) basal to the other retroviruses required at least an additional 17 steps over the shortest tree in maximum parsimony analysis (data not shown). These points imply that the presence of HERV.I-related viruses in sharks may be the result of horizontal transfer from another vertebrate class. However, numerous additional members of this group will have to be identified and characterized before their mode of dispersal within vertebrates can be definitively answered.



We screened four other (more primitive) vertebrate and chordate classes for the presence of endogenous retroviruses without success (data not shown). However, the number of taxa investigated has so far been small; DNA samples were available from only two species of lamprey (Cephalaspidomorphi, Petromyzoniformes), one hagfish (Myxini, Myxiniformes), one lancelet (Cephalochordata, Branchiostomidae), and one sea squirt (Urochordata, Ascidiacea), and investigation of a much larger number of primitive chordates is required before the absence of retroviruses in these groups can be considered probable. Furthermore, there is some evidence to suggest that retroviruses may be present in nonvertebrate/chordate taxa: seasonal neoplasm in the soft-shelled clam (*Mya arenaria*) has been linked to exogenous type B retroviruslike particles identified in this species (27, 28). However, PCR screening of nine species of mollusc (including the soft-shelled clam) failed to isolate any endogenous retroviral sequences (data not shown).

Retroviral phylogenies have been reported previously by a number of workers (6–8, 45). Many were constructed using amino acid data sets derived from the 5' end of the reverse transcriptase protein, and the PCR-amplified retroviral fragments described here contain most of this region. Phylogenetic reconstruction has generally resulted (when the trees are rooted) in the placement of the spumaviruses (here shown within group I) and MLVs (within group III) as the sister taxon to a (group II) lineage containing the other five retroviral

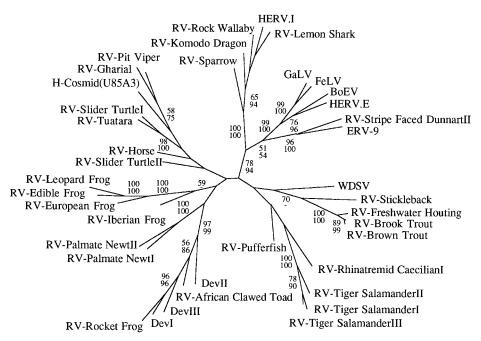


FIG. 4. Unrooted maximum parsimony tree of the group III viruses shown in Fig. 3. Branch lengths are proportional to the number of changes required to generate the observed variation. Numbers on each branch reflect percentage bootstrap support using maximum parsimony (upper value) or neighbor-joining (lower value).

genera (lentiviruses, HTLV-BLV group, ALVs, and type B and D retroviruses). Our phylogenetic analyses are consistent with this view of retroviral phylogeny but highlight the uncertainty of the interrelationships of the group I spumaviruslike sequences and the root of the retrovirus tree.

Despite screening a large number of lower vertebrate taxa, no endogenous retroviral sequences were identified which clustered with the lentiviruses (equine infectious anemia virus [EIAV], ovine maedi-visna virus [OMVV], and simian immunodeficiency virus SIVmac), the HTLV-BLV group, or the ALVs (Rous sarcoma virus [RSV]). Indeed, the only sequences which were placed with other group II viruses were basal to the murine type B (mouse mammary tumor virus [MMTV]) and mammalian type D (jaagsiekte and simian retrovirus type 1 [SRV1]) retroviruses, being placed in a polytomy with HERV. K10, RSV, lymphoproliferative disease virus (LDV), and intracisternal type-A retroviral particle (IAP-hamster). The absence of isolates derived from nonmammalian or avian hosts suggests that endogenous retroviruses related to these five genera are likely to be rare in lower vertebrate genomes. We think it unlikely that this distribution is simply the result of target sequences being missed during amplification for several reasons. (i) The EM primer was designed against a sequence motif conserved in each of the five genera, (ii) negative results using EM were obtained with more than 30 species of reptiles, amphibians, and fish, (iii) the EM primer gave positive results with mammalian and avian taxa, and (iv) the JO and CT primers have previously cross-amplified group II-related viruses from mammalian genomes (37) but failed to do so when used against lower vertebrate taxa. The distribution of the group II sequences in mammals and birds is interesting, particularly given their apparent absence from reptilian relatives of birds. This suggests that their distribution may involve either dispersal across significant taxonomic distances or possibly the extinction of some taxa.

The spumaviruses (human spumavirus [HSV], two simian foamy viruses [SFV and SFVL3], and FeSFV) are exogenous

viruses of primates and other mammals (4). There have been previous reports of two endogenous retroviruses which cluster with this genus, SpeV from the reptile tuatara (39) and HERV.L, a recently identified human isolate (5). It has been suggested that HERV.L represents a possible ancestor of the exogenous mammalian spumaviruses (5). Our phylogenetic analyses suggest that viruses distantly related to the spumaviruses may be widespread in vertebrates: unrooted trees (Fig. 3a) showed strong support for a group containing the spumaviruses and novel isolates derived from birds, reptiles, amphibians, and fish. However, the spumaviruses are unlikely to have emerged from HERV.L or a closely related endogenous virus, because this element appears more closely related to several viruses derived from nonmammalian hosts. The piscine member of the spumaviruslike lineage, SnRV, is an exogenous retrovirus which was originally isolated from striped snakehead fish (10). It has previously been reported to group (albeit distantly) with the MLVs (13), but the inclusion of additional retroviral isolates (which were not available to previous workers) suggests this may not be correct. It is our opinion, however, that none of the viruses described here should be assigned to the spumavirus genus. Although a spumaviruslike group was well supported by bootstrap analysis in unrooted phylogenies, this was not the case when several gypsy LTR retrotransposon sequences were included for rooting purposes (Fig. 3b). Furthermore, the monophyly of this group within individual rooted neighbor-joining and maximum parsimony trees (Fig. 3c and d) was outgroup dependent; varying the composition of the outgroup taxa sometimes resulted in the placement of the spumaviruslike viruses into two or three separate groups. These factors suggest that the spumavirus genus is only distantly related to the other group I viruses described here. Consistent with this, SnRV, HERV.L, and the spumaviruses are known to have significant differences in genomic organization when compared to each other and to other retroviral genera (5, 13). We were also unable to detect obvious

sequence homology between the accessory proteins of SnRV and those encoded by the spumaviruses (data not shown).

The vast majority of endogenous lower vertebrate viruses characterized during this study (group III) clustered with the MLV-related genus. MLV-like viruses are present in numerous mammalian species and have also been described in birds (such as the spleen necrosis virus [SNV] and reticuloendotheliosis virus [REV] of domestic fowl) and several reptiles, including the corn snake and Russell's viper (3, 19, 29, 31, 48). Several other groups of endogenous viruses (such as the HERV.I-related viruses and ERV-9 [also present within humans]) cluster with these viruses (17, 21, 44). Thus, members of this retroviral genus are already known to be present within several vertebrate classes. However, the phylogenetic trees shown in Fig. 3 demonstrate that the lineage containing the MLV genus is extremely complex. A polytomy, consisting of 13 lineages (one of which contained MLV/ERV-9/HERV.I-related viruses), was left unresolved by bootstrap analyses. Several of these lineages contained a single retroviral sequence, such as the previously described WDSV of fish (15), whereas others contained groups with several members. We believe that many of these lineages are likely to represent novel retroviral genera. Although other factors, in addition to sequence divergence (such as the presence of novel genes and alternative primer binding site homology), are required for genus-level classification (4), these may well become apparent when the complete nucleotide sequences of these viruses are determined. For example, it has already been suggested that WDSV represents a novel retroviral genus because it encodes two accessory proteins which are absent from members of the MLV genus and (unusually) utilizes a primer binding site homologous to tRNA<sup>His</sup> (15, 23).

Although most of the retroviruses clustering in this region of the tree (excluding those present within the MLV genus) were derived from reptiles, amphibians, and fish, it was apparent that two novel mammalian viruses were also present. We originally isolated the first of these (RV-horse) when screening mammalian genomes but were unable at the time to determine its phylogenetic relationship to other retroviruses. The second element, H-cosmid(U85A3), was identified from a BLAST search of the EMBL/GenBank/DDBJ data banks and was originally sequenced as part of the human genome project (25). Our sequence analysis of this element suggests it contains a full-length polymerase gene, part of the envelope region, and that the gag gene has probably been replaced by a later insertion (in the reverse orientation) of an HERV-H-like element (unpublished results). We also identified a second element (by BLAST searches), closely related to H-cosmid(U85A3) on the X chromosome within cosmid HS49L23 (12). These results imply that other highly divergent endogenous retroviruses remain to be discovered in both humans and other mammals.

Finally, the distribution and phylogeny of the sequences shown in Fig. 3 (especially those within group III) is intriguing as it suggests that some of the retroviral groups may be restricted to particular vertebrate classes, indicating that interclass horizontal transmission may be a rare event for certain types of retrovirus. Although interpretation of these trees should be cautious (due to the lack of robust support for many of the relationships), the overall tree topologies are consistent with this possibility. For example, the majority of endogenous piscine retroviruses appear to be monophyletic, even though their hosts are classified into several different taxonomic orders. This also appears to be the case for many of the reptilian and amphibian retroviruses. Tests using the program Mac-Clade also demonstrated that the host class-virus associations shown in Fig. 4 are phylogenetically correlated. We are currently trying to obtain a better idea of interclass transmission rates by looking in detail at the phylogeny and distribution of the MLV genus.

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