Novel Amdovirus in Gray Foxes

Linlin Li, Patricia A. Pesavento, Leslie Woods, Deana L. Clifford, Jennifer Luff, Chunlin Wang, and Eric Delwart

We used viral metagenomics to identify a novel parvovirus in tissues of a gray fox (*Urocyon cinereoargenteus*). Nearly full genome characterization and phylogenetic analyses showed this parvovirus (provisionally named gray fox amdovirus) to be distantly related to Aleutian mink disease virus, representing the second viral species in the *Amdovirus* genus.

Aleutian mink disease virus (AMDV) is currently the only member of the genus *Amdovirus* in the family *Parvoviridae*; it can infect diverse breeds of farmed and feral mink, in addition to other mustelids (e.g., ferrets, otters), raccoons, and foxes (*1*,*2*). AMDV has an ≈5-kb single-stranded DNA genome and, like other parvoviruses, replicates through a rolling-hairpin mechanism (*3*). The viral genome has 2 large open reading frames (ORFs), encoding nonstructural (NS1, NS2, putative NS3) and structural viral proteins (VP1 and VP2). Alternative splicing enables expression of multiple messenger RNAs (*4*). AMDV strains can exhibit sequence variability in their NS gene, and 3 genetic groups have been identified on the basis of partial nucleotide sequences of this region (*5*).

AMDV infection can cause an acute and fatal interstitial pneumonia in newborn mink. It can also cause a chronic disorder of the immune system in adult mink, characterized by persistent viral infection, plasmacytosis, hypergammaglobulinemia, and immune complex–mediated glomerulonephritis and arteritis, resulting in major economic losses to mink farms (*6*). AMDV infection can also be asymptomatic. The different outcomes are determined by host factors that include age, immune status, and the virulence of the virus strains (*7*,*8*). AMDV can be transmitted through urine, feces, and saliva as well as vertically in utero (*9*,*10*). In 1 report, a ferret found to be naturally infected with AMDV showed acute dyspnea and posterior paresis with histopathologic lesions similar to those seen in mink; the ferret became comatose and died (*11*). Recently, 2 mink farmers with vascular disease and microangiopathy, similar to conditions in mink with Aleutian disease, were found to have AMDV-specific antibodies and were AMDV DNA positive, suggesting a potential relationship between AMDV and human symptoms (*12*).

We used random PCR amplification and high-throughput sequencing technology to investigate viral sequences found in the spleen and lung tissues of a sick gray fox (*Urocyon cinereoargenteus*) from California. A highly divergent amdovirus was identified, and the near full genome of this virus was obtained. Phylogenetic analysis indicated that this virus, designated as gray fox amdovirus, is a new amdovirus species, only the second for that genus.

The Study

The gray fox studied here was identified during the summer of 2009 in Sonoma County, California. It had severe gait abnormalities, lymphadenopathy, and acute muscle inflammation, and was euthanized at a wildlife rehabilitation center. Using the generic viral particle enrichment method previously described for tissues (*13*), we generated ≈14,000 sequence reads from spleen and lung samples. We found 136 sequence reads in spleen tissue that were related to AMDV by using BLASTx (E score $<10^{-5}$) (www.ncbi.nlm.nih.gov/BLAST); these could be assembled into 24 contigs covering ≈60% of the viral genome. By connecting gaps between sequenced viral fragments and amplifying the genome extremities by using PCR primers based on AMDV sequences, the nearly complete genome of the new amdovirus (GenBank accession no. JN202450) was acquired. We temporarily named it gray fox amdovirus (GFADV).

The partial GFADV genome was 4,441 nt in length with a low guanine–cytosine content of 37%. Similar to that of AMDV, the GFADV genome contained 2 major ORFs. The left ORF (LORF) contains the bulk of the sequences for the putative NS1, and the right ORF (RORF) codes for VP2 (Figure 1). Two small middle ORFs (67 and 75 aa long) with putative alternative start codons were detected in the 448-bp region between the ORFs. The theoretical proteins showed 55% and 59% aa identity with the 2 similarly located middle ORFs reported in AMDV. The partial 5′ untranslated region (UTR) was 109 nt and the partial 3′ UTR was 191 nt. Potential RNA splicing signals in AMDV were present on the GFADV genome (online Technical Appendix Figure 1, www.cdc.gov/EID/content/17/10/11-0233-Techapp.pdf) (*4*). The predicted spliced transcripts encode hypothetical NS1, NS2, and NS3 of 635 aa, 115 aa, and 80 aa, respectively, and a capsid protein VP1 of 674 aa. The putative VP2

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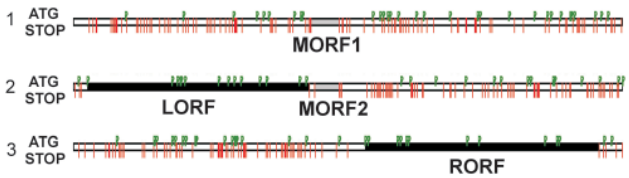

Figure 1. Open reading frames (ORFs) in gray fox amdovirus genome. Three possible reading frames of the plus-strand sequence with the stop codons indicated by red lines and ATG codons by green flags. Two major ORFs, left (LORF) and right (RORF), are indicated by black bars; 2 small middle ORFs (MORF1 and MORF2) are indicated by gray bars.

was predicted to arise from the intact transcript of RORF encoding a 630-aa protein.

Sequence analyses confirmed that GFADV was a divergent amdovirus with 76% nt identity with the genome of AMDV. Conserved protein domains typical of parvoviruses were identified in GFADV. In the LORF, the GKRN domain was found (online Technical Appendix Figure 2), which may act as the nuclear transport signal of NS1 protein. In the RORF, 3 conserved domains (TPW, YNN, and PIW) of unknown biologic significance were detected (*6*,*14*) (online Technical Appendix Figure 3). The phosholipase 2 motif in the N terminal VP1 region, generally conserved in parvoviruses, was not found in either GFADV not AMDV (online Appendix Figure 3), which suggests that this parvovirus genus uses a different mechanism to escape the endosome during infection (*15*).

Comparison of NS1 regions showed GFADV shared ≈74% nt and 67% aa similarities with AMDV strains, whereas different strains of AMDV shared >87% nt and 82% aa similarities. Alignments of the VP2 region showed GFADV shared ≈78% nt and 80% aa similarities with AMDV strains, whereas strains of AMDV had >92% nt and 91% aa similarities (online Technical Appendix Figures 4, 5). To determine the relationship between GFADV and AMDV strains, phylogenetic analyses of the NS1 and VP2 proteins were performed, which showed that in both genome regions GFADV was more closely related to AMDV strains than to those of minute virus of mice or other parvoviruses analyzed (data not shown), but was distinct from the 3 AMDV groups (Figure 2). Pending review by the International Committee on Taxonomy of Viruses, GFADV thus appears to be the second reported parvovirus species in the genus *Amdovirus*.

GFADV sequences were also detected in the lung and heart tissues of the same animal by using a GFADV-specific nested PCR targeting a ≈400-bp segment of the VP2 gene, as well as in the heart tissue of another gray fox, which had the same signs, collected in Sonoma County in summer 2009. Further PCR screening of 19 tissue samples, including spleen, lung, liver, lymph node, and muscle from 9 other gray foxes with similar gait abnormalities and chronic muscle lesions, collected in 2008 (n = 2) and 2010 (n = 7) were negative by the same GFADV PCR.

Conclusions

We report the identification and nearly complete genome sequence of an amdovirus found in the spleen, lung, and heart tissues of 2 gray foxes that exhibited an abnormal gait and muscle inflammation of unknown origin. On the basis of phylogenetic analyses, we propose this virus as the prototype member of a second species in the *Parvoviridae* genus *Amdovirus*. Putative NS and VP1/VP2 gene RNA splicing sites were detected in the GFADV genome, which suggests the expression of different NS and VP proteins.

Except for the ubiquitous anellovirus, GFADV was the only eukaryotic virus found in the spleen tissue of the diseased gray fox. The same virus was also identified in the heart tissue of a second gray fox collected the same summer but was not detected in tissues of 9 other gray foxes with a similar syndrome collected in different years. It is possible that GFADV is an incidental finding unrelated to these foxes' symptoms. The lack of detectable GFADV DNA in all gray foxes with similar symptoms may also be because different tissues were compared in some animals or because tissue collection occurred at different stage of infection. Future testing of a possible link between GFADV and additional unexplained diseases of foxes and

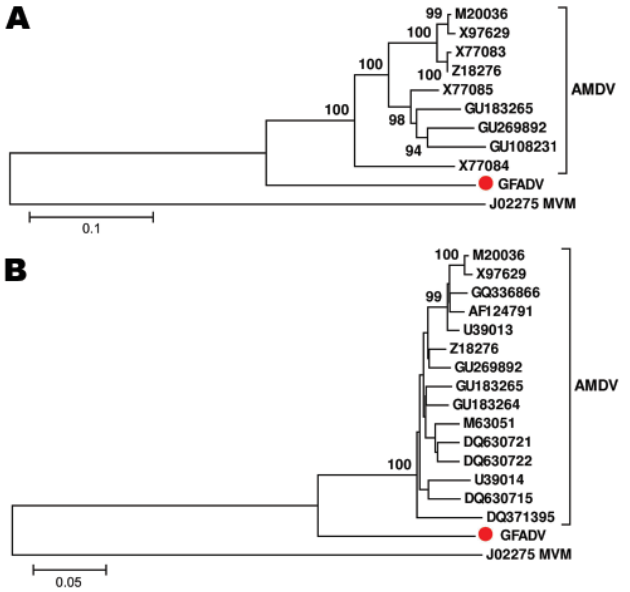

Figure 2. Phylogenetic analyses of gray fox amdovirus (GFADV) (red dots) and Aleutian mink disease virus (AMDV) based on the complete amino acid sequence of nonstructural protein 1 region (A) and viral protein 1 region (B). The neighbor-joining method was used with *p*-distance and 1,000 bootstrap replicates. Scale bars represent estimated phylogenetic divergence. GenBank accession numbers are shown on the tree. Minute virus of mice (MVM) was included as an outgroup.

other carnivores will be facilitated by the availability of its genome sequence.

Acknowledgments

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Dr Li is a staff scientist at the Blood Systems Research Institute, San Francisco, California. Her research interests are infectious diseases and viral discovery.

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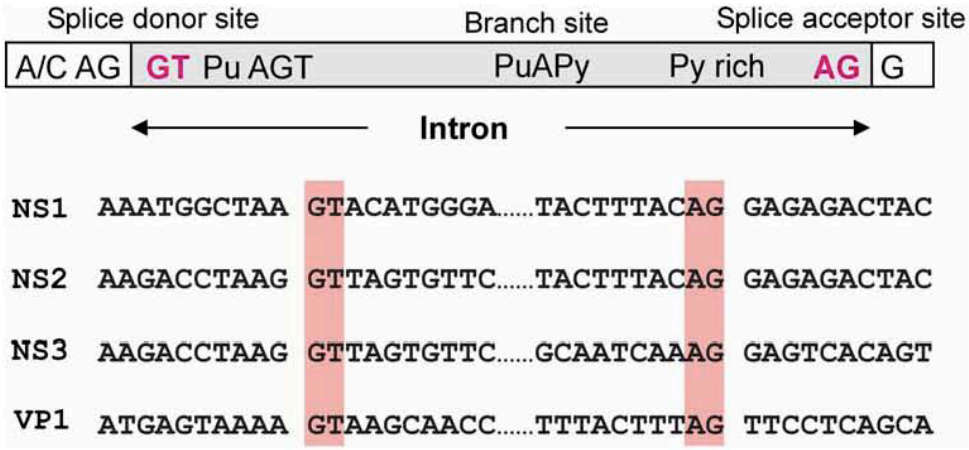
Technical Appendix



Technical Appendix Figure 1. Nucleotide sequence around the predicted RNA splicing sites in the gray fox amdovirus genome. Splice donor and receptor are shown in pink boxes. NS, nonstructural; VP, viral protein.

```
GFADV     1 MAQAQIAEQKILQALFEQLKKEIADGEGLAWLFQQKAYTDGDNKPTKETPPLRTTSEDLRLVFDSIEEN---------IK
AMDV      1 MAQAQIDEQRRLQDLYVQLKKEINDGEGVAWLFQQKTYTDKDNKPTKATPPLRTTSSDLRLAFDSIEEN---------LT
MVM       1 MISGSGSLNQGAKRKWAWFKVYKQLLKSVTYLFFHSVSRDAQKESNQLTMAGNAYSDEVLGATNWLKEKSNQEVFSFVFK
BPV       1 -MQPSRGLYSALHSFKHLLITIRQRTHAPDTVLSRDELNAWNWLQKIAQRVMRGDMDDIVDILKCRKAN-----------
AAV2      1 -----------MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSDMDLNLIEQAPLTVAEK-----------

GFADV    72 SNTDCLSNNEI---NFCKLTLGKTLVSLDKHIKSHRWNANNLQFIWQVEKGKTKHLHIHCLVG---YSDSTADKKDISKS
AMDV     72 ASNEHLTNNEI---NFCKLTLGKTLLLIDKHVKSHRWDSNKVNLIWQIEKGKTQQFHIHCCLG---YFDKNEDPKDVQKS
MVM      81 NENVQLNGKDIGWNSYKKELQEDELKSLQRGAETTWDQSEDMEWETTVDEMTKKQVFIFDSLVKKCLFEVLNTKNIFPGD
BPV      69 ------------------------------------------------------------------------------
AAV2     59 --------------------------LQRDFLTEWRRVS-----------------------------------KAP

GFADV   146 LSWFIKKINKELATIWSNHHCTLQDIKRPEDR--QQNLNVWLQDGITKPYKYFNKQTRQEYNKQVNLREYTLIYLFDKNK
AMDV    146 LGWFMKRLNKDLAVIYSNHHCDIQDIKDPEDR--AKNLKVWIEDGPTKPYKYFNKQTKQDYNKPVHLRDYTFIYLFNKDK
MVM     161 VNWFVQHEWGKDQGWHCHVLIGGKDFSQAQGKWWRRQLNVYWSRWLVTACNVQLTPAERIKLFEIAEDNEWVTLLTYKHK
BPV      69 -GTLVAQAINGTEFITRYMLPKNR-----------------------------KVADTVLTRHTTPEQ--------SYD
AAV2     75 EALFFVQFEKGESYFHMHVLVETTGVKSMVLG------------------RFLSQIREKLIQRIYRGIEPTLPNWFAVTK

GFADV   224 ITEKGMDGYFAAGNGGFIDNMTEKERKIMRKMYLDEQCSDMADDSQDWDDSQEPPKTEPPKVTTETISNVTYVDSAVVQP
AMDV    224 INTDSMDGYFAAGNGGIVDNLTNKERKTLRKMYLDEQSSDIMDANIDWEDGQ-----DAPKVTDQT-------DSATTKT
MVM     241 QTKKDYTKCVLFGN--MIAYYFLTKKKISTSPPRD--GGYFLSSDSGWKTNF---------LKEGE-------RHLVSKL
BPV     110 STWGKTYGFAVCNGETVSEFTRKDLWKVLYNIYTAHPAENMLNSN-------------------------------PSV
AAV2    137 TRNGAGGGNKVVDECYIPNYLLPKTQPELQWAWTN--MEQYLSACLNLTERK-----------------------RLVAQ

GFADV   304 GTSTIWASCSTKATKPKTITETAKQPSKKLTTAKSTLDSLFDIGCFTPEDMILKLSDTYLELSLEANGTNKILTLLHMNQ
AMDV    292 GTSLIWKSCATKVTSKKEVANPVQQPSKKLYSAQSTLDALFNVGCFTPEDMIIKQSDKYLELSLEPNGPQKINTLLHMNQ
MVM     301 YTDDMRPETVETTVTTAQETKRGRIQTKKEVSIKTTLKELVHKRVTSPEDWMMMQPDSYIEMMAQPGGENLLKNTLEICT
BPV     158 WGDLPRVSANRIDADDAEARSRPIKLSRKQKIMAEVIQRATDGLLLTYNDLVVHLSDLMLMLEGMPGGSKTAEQLLTMIH
AAV2    192 HLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEKQWIQEDQASYISFNAASNSRSQIKAALDNAG

GFADV   384 VKTASILNAFECIMKFNDDE------------DEKPLINIINQMGLNEKVLKNIIATVLTKQSGKRGCIWFYGPGGTGKT
AMDV    372 VKTSTMITAFDCIIKFNEEE------------DDKPLLATIKDMGLNEQYLKKVLCTILTKQGGKRGCIWFYGPGGTGKT
MVM     381 LTLARTKTAFDLILEKAETSKLT-----NFSLPDTRTCRIFAFHGWNYVKVCHAICCVLNRQGGKRNTVLFHGPASTGKS
BPV     238 IKLCAKYNAYEFMLMKTPATQNMNPGAPHYDCQGNLVFKLLNLQGYNPWQVGHWLVMMLSKKTGKRNSTLFYGPASTGKT
AAV2    272 KIMSLTKTAPDYLVGQQPVED----------ISSNRIYKILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKT

GFADV   452 LLANLICTAVKNFGMVTTSNQNFPWTDCGNRNMIWLEECGNLGNFIEDFKAITGGGDIKVDTKNKQPQAIKG-VTVITSN
AMDV    440 LLASLICKATVNYGMVTTSNPNFPWTDCGNRNIIWAEECGNFGNWVEDFKAITGGGDVKVDTKNKQPQSIKG-CVIVTSN
MVM     456 IIAQAIAQAVGNVGCYNAANVNFPFNDCTNKNLIWVEEAGNFGQQVNQFKAICSGQTIRIDQKGKGSKQIEPTPVIMTTN
BPV     318 NLAKAICHAVGLYGCVNHNNKQFPFNDAPNKMILWWEECIMTTDYVEAAKCVLGGTHVRVDVKHKDSRELPQIPVLLSSN
AAV2    342 NIAEAIAHTVPFYGCVNWTNENFPFNDCVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSN

GFADV   531 KDITKVTIGAVETNVHSEPLKQRIVKIRCVKTINPTTKITP---------------------------------------
AMDV    519 TNITKVTVGCVETNAHAEPLKQRMIKIRCMKTINPKTKITP---------------------------------------
MVM     536 ENITVVRIGCEERPEHTQPIRDRMLNIHLTHTLPGDFGLVDK-----------------NEWPMICAWLVKNGYQSTMAS
BPV     398 HDVYTVVGGNATFGVHAAPLKERITQMNFMKQLPNTFGEITPGMISNWLSHCAHIHQEHLSLEGFAIKWDVQSVGNSFPL
AAV2    422 TNMCAVIDGNSTTFEHQQPLQDRMPKFELTRRLDHDFGKVTK-----------------QEVKDFFRWAKDHVVEVEHEF

GFADV   572 -----GMLKKW--------------------LSTWEG----KPIQLNHEMENLYRSK----------------------
AMDV    560 -----GMLKRW--------------------LNTWDR----QPIQLSHEMPELYLGKCRW--------------------
MVM     599 YCAKWGKVPDW--------------------SENWAEPKVPTPINLLGSARSPFTTPKSTPLSQN---------------
BPV     478 QTLCPGHSQNWTFSENGVCWHCGGFIQPTPESDTDSDGDPDPDGAVAGDSDTSANSESTVSFSSNDSGLGSVTSSAPSVP
AAV2    485 YVKKGGAKKRP--------------------APSDADISEPKRVRESVAQPSTSDAEASINYADR---------------

GFADV       ------------------------------------------------------------------------------
AMDV        ------------------------------------------------------------------------------
MVM     644 --------------------------YALTPLASDLEDLALEPWS------------TPNTPVAGTAETQNTGEAGSKAC
BPV     558 DRAEEIEEIPSECLEWMREEVDRLSAHDINSLAHQATGFILDPIPEEPEEGERDLAREDAEPEASTSHTPATKRARVEEG
AAV2    530 --------------------------YQNKCSRHVGMNLMLFPCR------------QCERMNQNSNICFTHGQKDCLEC

GFADV       ------------------------------------------------------------------------------
AMDV        ------------------------------------------------------------------------------
MVM     686 QDGQLSPTWSEIEEDLR--------------ACFGAEPLKKDFSEPLNLD------------------------------
BPV     638 EPWDGTQPITEGDWIDFESRQKRRRLEREEKGGEDEDMEVQESDPSAWGEKLGIVEKPGEEPIVLYCFETLPESDEEGDS
AAV2    572 FPVSESQPVSVVKKAYQKLCYIHHIMGKVPDACTACDLVNVDLDDCIFEQ-------------------------------

GFADV       ---------
AMDV        ---------
MVM         ---------
BPV     718 DKENKTHTV
AAV2        ---------
```

Technical Appendix Figure 2. Conservative domain in the left open reading frame of gray fox amdovirus and representative parvoviruses. GKRN region is marked in red.

```
GFADV    1 ------------------------------------------------------------------------------------
AMDV     1 ------------------------------------------------------------------------------------
MVM      1 --------------------------------MAPPAKR-AKRGWVPPGYKYLGPGNSLDQGEPTNPSDAAAKEHDEAYD
BPV      1 --------------------------------MPPTNKANSKKGLTLPGYNYLGPFNSLFAGAPVNKADAAARKHDFGYS
AAV2     1 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKGEPVNEADAAALEHDKAYD

GFADV    1 ------------MSKIPQHYP-----------------------------------------GKKRSAPRHVFIQQAK-
AMDV     1 HHNTLLSLYLCYFTLVPQHYP-----------------------------------------GKKRSAPRHVFIQQAK-
MVM     48 QYIKSGKNPYLYFSAADQRFIDQTKDAKDWGGKVGHYFFRTKRAFAPKLATDSEPGTSGVSRAGKRTRPPAYIFINQAR-
BPV     49 DLLKEGKNPYLYFNTHDQNLIDELKDDTSFGGKLARGVFQIKKALAPALPG--------TSKGGDRALKRKLYFARSNKG
AAV2    81 RQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEP--VKTAPGKKRPVEHSPVEPDSSSG

GFADV   26 KKKQHSNPAVSHQEDTIEEMDSNETEQMDISEQQ-QVAEHGGG-------------VGKSTGGFNNTTEFKVTNN-EVII
AMDV    38 KKKQ-TNPAVYHGEDTIEEMDSTEAEQMDTEQATNQTAEAGGGGGGGGGGGGGGGVGNSTGGFNNTTEFKVINN-EVYI
MVM    127 AKKKLTSSAAQQSSQTMSDG-TSQPDSGNAVHSAARVERAADGPGGSGGGGSGGGGVGVSTGSYDNQTHYRFLGDGWVEI
BPV    121 AKKANREPAPSTSNQQNMEVSNDIPNDEAGNQPIELATRSVVGSGSVGGGGRGGSGVGYSTGGWTGGTIFS------ENI
AAV2   159 TGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDR

GFADV   91 TCNATRVVHINQASTDEYLIFNAGREID-KTPKGSLNLEFFMFDDPHQQVMTPWFLVDSNAWGVWMSPKDFQQMKVLCSE
AMDV   116 TCHATRMVHINQADTDEYLIFNAGRTTDTKTHQQKLNLEFFVYDDPHQQVMTPWYIVDSNAWGVWMSPKDFQQMKTLCSE
MVM    206 TALATRLVHLNMPKSENYCRIRVHNTTDTSVKGN------MAKDDAHEQIWTPWSLVDANAWGVWLQPSDWQYICNTMSQ
BPV    195 VVTKNTRQFTCDIKNGHLYKSEVLNTGDTAHRQY--------------AITTPWSYFNFNQYSSHFSPNDWQHLVNDYER
AAV2   239 VITTSTRTWALPTYNNHLYKQISSQSGASNDNHY-------------FGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWG

GFADV  170 IELVSLEQEIDNVVIKTVTETTQGGTSVIQYNNDLTASLQVALDTNNILPYTPAAPLQETLGFVPWRATKPTQYRYYHPS
AMDV   196 ISLVTLEQEIDNVTIKTVTETNQGNASTKQFNNDLTASLQVALDTNNILPYTPAAPLGETLGFVPWRATKPTQYRYYHPC
MVM    280 LNLVSLDQEIFNVVLKTVTEQDLGGQAIKIYNNDLTACMMVAVDSNNILPYTPAANSMETLGFYPWKPTIASPYRYY---
BPV    261 FRPKAMIVRVYNLQIKQIMTD---GAMGTVYNNDLTAGMHIFCDGDHRYPYVQHPWDDQCMPELPNSIWELPQYAYIPAP
AAV2   306 FRPKRLNFKLFNIQVKEVTQN----DGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLN

GFADV  250 YIYNRYPNIQKKSQEQSTYTAIQDDYFSVDQDYFNFITIENNIPIEILRTGDNFHTGRYEFKSKPCKLTMSYQSTRCLGL
AMDV   276 YIYNRYPNIQKVATETLTWDAVQDDYLSVDEQYFNFITIENNIPINILRTGDNFHTGLYEFNSKPCKLTLSYQSTRCLGL
MVM    357 FCVDRDLSVTYENQEGTVEHNVMGTPKGMNSQFF---TIENTQQITLLRTGDEFATGTYYFDTNSVKLTHTWQTNRQLGQ
BPV    338 ISVVDN-NTTNTVEEHLLKGVPLYMLENSDHEVLRNGRIYR-IYIQLWRLRMDRKQ--HHIQHASDDVQSTGQKQKNLLI
AAV2   382 NGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQ

GFADV  330 PPLCKPKTDT-KLVTSL--ENGGEMDCYVEGQQHTRKGHFWGEEKAEKNTEINRVRPYNIGYQFPEWIVPAGLQGSYFTG
AMDV   356 PPLCKPKTDTTHKVTSK--ENGADL-IYIQGQDNTRLGHFWGEERGKKNAEMNRIRPYNIGYQYPEWIIPAGLQGSYFAG
MVM    434 PPLLSTFPEADTDAGTLTAQGSR--------HGTTQMGVNWVS-------EAIRTRPAQVGFCQPHNDFEASRAGPFAAP
BPV    414 QRTKQPNKQRFQNAALR-------------------------------TSNWMSGPGIARGTHNATLQTQSAGALVTM
AAV2   462 FSQAGASDIRDQSRNWLP----------------------------GPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGR

GFADV  407 GPRQWSDTTKGAGTHSQTVQQNFSTRYVFDRNHGGDNQVDTIDSIPIQERGKYYSEQELQQATTLTATNKAQ--TGKKWE
AMDV   433 GPRQWSDTTKGAGTHSQHLQQNFSTRYIYDRNHGGDNEVDLLDGIPIHERSNYYSDNEIEQHTAKQPKLRTPPIHHSKID
MVM    499 -------KVPADITQGVDKEANGSVRYSYGKQHGEN----WASHGPAPER---YTWDETSFGSGRD-------------
BPV    461 V------TNGADVSGVRAVRVGYSTDPIYGGQQPESDLLRLRYSASAAEG-------------------------------
AAV2   514 DSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEE-------------------------------

GFADV  485 SWEEEGWPAASGTHFEDEVLYFDYFNFSGETTKNFPQEVLSDGLQMKKLLNAYQPTVGQDDVGPVYPWGQIWDKKPDMDH
AMDV   513 SWEEEGWPAASGTHFEDEVIYLDYFNFSGEQELNFPHEVLDDAAQMKKLLNSYQPTVAQDNVGPVYPWGQIWDKKPHMDH
MVM    551 ----------TKDGFIQSAPLVVPPPLNGILTNANPIGTKND-IHFSNVFNSYGPLTAFSHPSPVYPQGQIWDKELDLEH
BPV    505 ----------QQNPILENAARHTFTREARTKLITGSNGADGDYKEWWMLPN------QMWDSAPISRYNPIWVKVPRVNR
AAV2   564 -----------EIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPG------MVWQDRDVYLQGPIWAKIPHTDG

GFADV  565 KPSMNYNAPFVCKNNPPGQLLVKLTENLTDKFN-YDEDP--DRIKTYGYFTWRGKLTLRGKLGQTSCWNPVKRERIGEPG
AMDV   593 KPSMNNNAPFVCKNNPPGQLFVKLTENLTDTFN-YDENP--DRIKTYGYFTWRGKLVLKGKLSQVTCWNPVKRELIGEPG
MVM    620 KPRLHITAPFVCKNNAPGQMLVRLGPNLTDQYDPNGATL--SRIVTYGTFFWKGKLTMRAKLRANTTWNPVYQVSAEDNG
BPV    569 KTLLDTQDGSIPMSHPPGTIFIKLAR-----IPVPGNGD--SFLNIYVTGQVSCEVVWEVEKRGTKNWRPEYMHSATNMS
AAV2   627 HFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSN---

GFADV  642 VFSKSNYHKQIPNNRGNFEIGIQYGRSTVKYLY
AMDV   670 VFTKDKYHKQIPNNKGNFEIGLQYGRSTIKYIY
MVM    698 NSYMS-VTKWLPTATGNMQSVPLITRPVARNTY
BPV    642 VDAYTINNAGVYAGAVQNADVMQTRFNHHKVL-
AAV2   704 YNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL-
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Technical Appendix Figure 3. Conservative domains in the right open reading frame of gray fox amdovirus and representative parvoviruses. PGY region (putative phopholipases A2 domain) is marked in blue and TPW, YNN, and PIW region are marked in red.

```
GFADV      1 MAQAQIAEQKILQALFEQLKKEIADGEGLAWLFQQKAYTDGDNKPTKETPPLRTTSEDLRLVFDSIEENIKSNTDCLSNN
M20036     1 MAQAQIDEQRRLQDLYVQLKKEINDGEGVAWLFQQKTYTDKDNKPTKATPPLRTTSSDLRLAFDSIEENLTASNEHLTNN
X77084     1 MAQAQLNEQRRLQELFEKFKTEVADGEGLAWLFQQKTYTDKDNKPTKATPPLRTTSTDLRLAFDSIEETLKTSNQCLTNN
X77085     1 MAQAQLDEQKRLQDLYTQLKKEVADGEGLAWLFQQKTYTDKDNKPTKATPPLRTTSSDLRLAFDSIEETLIASNEWLTKD

GFADV     81 EINFCKLTLGKTLVSLDKHIKSHRWNANNLQFIWQVEKGKTKHLHIHCLVGYSDSTADKKDISKSLSWFIKKINKELATI
M20036    81 EINFCKLTLGKTLLLIDKHVKSHRWDSNKVNLIWQIEKGKTQQFHIHCCLGYFDKNEDPKDVQKSLGWFMKRLNKDLAVI
X77084    81 DINFCKLTLGKTLVLLDKHVRSHRWDANKVNFIWQVEKGKTQQFHIHCCLGYFDKDEDSKDVQKSLGWFIKKLNKDLAVI
X77085    81 EINFCKLTLGKTLVLVDKHVKSHRWDADKINFIWQIEKGKTQHFHIHCCLGYFDKNEDPKDVQKSLGWLIKKLNRDLAAI

GFADV    161 WSNHHCTLQDIKRPEDRQQNLNVWLQDGITKPYKYFNKQTRQEYNKQVNLREYTLIYLFDKNKITEKGMDGYFAAGNGGF
M20036   161 YSNHHCDIQDIKDPEDRAKNLKVWIEDGPTKPYKYFNKQTKQDYNKPVHLRDYTFIYLFNKDKINTDSMDGYFAAGNGGI
X77084   161 WSNHHCDIQGIQDSDGRADNLKIWIEDGPTKPYKYFNKQTKQDYNKPTNLRDYTLIYLFNKDKITQEGMDGYYASGNGGI
X77085   161 FSNHHCDQQDIKDPEAKANNLKVWIEDGPTKPYKYYNKQTKQDYNKPVTLSDYVIIYLFNKDKIHKEGMDGYYAAGNGGL

GFADV    241 IDNMTEKERKIMRKMYLDEQCSDMADDSQDWDDSQEPPKTEPPKVTTETISNVTYVDSAVVQPGTSTIWASCSTKATKPK
M20036   241 VDNLTNKERKTLRKMYLDEQSSDIMDANIDWEDGQD-----APKVTDQT-------DSATTKTGTSLIWKSCATKVTSKK
X77084   241 IDNLTNKERKALRKMYLDEQSQGILDEDIDWEDSQS-----APKVTDQT-------DSATSKTGTSLVWKSCATKVTSKK
X77085   241 IDNLTNKERKALRKMYLDEQSSDIMDADIDWEDGQD-----APKVTDQT-------DSATSKTGTSLIWKSCATKVTSKK

GFADV    321 TITETAKQPSKKLTTAKSTLDSLFDIGCFTPEDMILKLSDTYLELSLEANGTNKILTLLHMNQVKTASILNAFECIMKFN
M20036   309 EVANPVQQPSKKLYSAQSTLDALFNVGCFTPEDMIIKQSDKYLELSLEPNGPQKINTLLHMNQVKTSTMITAFDCIIKFN
X77084   309 EVAIPVKQPSKKMTSAQNTLDDLYMFGCFTPEDMILKMSDRYLEMSLEPNGAQKINTLLHMNQVRTSSNLTAFECIIKFN
X77085   309 EVANPVQQPSKKLYSAQNTLDALFNVGCFTPEDMIIKQSDKYLELSLEPNGPQKINTLLHMNQVKTSTMMTAFDCIIKFN

GFADV    401 DDEDEKPLINIINQMGLNEKVLKNIIATVLTKQSGKRGCIWFYGPGGTGKTLLANLICTAVKNFGMVTTSNQNFPWTDCG
M20036   389 EEEDDKPLLATIKDMGLNEQYLKKVLCTILTKQGGKRGCIWFYGPGGTGKTLLASLICKATVNYGMVTTSNPNFPWTDCG
X77084   389 EEEDEKPLLDTIKDMGLNEQHLKKVLCTILTKQSGKRGCVWFYGPGGTGKTLLASLICKAVVNYGMVTTSNPNFPWTDCG
X77085   389 EEEDDKPLLATIKDMGLNEQYLKKVLCTILTKQGGKRGCIWFYGPGGTGKTLLASLICKATVNYGMVTTSNPNFPWTDCG

GFADV    481 NRNMIWLEECGNLGNFIEDFKAITGGGDIKVDTKNKQPQAIKGVTVITSNKDITKVTIGAVETNVHSEPLKQRIVKIRCV
M20036   469 NRNIIWAEECGNFGNWVEDFKAITGGGDVKVDTKNKQPQSIKGCVIVTSNTNITKVTVGCVETNAHAEPLKQRMIKIRCM
X77084   469 NRNIIWAEECGNIGNYVEDFKAITGGGDVKVDTKNKQPQSIKGSVIVTSNTNITKVTVGCVETNVHAEPLKQRMVKIRCM
X77085   469 NRNIIWAEECGNLGNWVEDFKAITGGGDVKVDTKNKQPQSIKGCVIVTSNTNITKVTVGCVETNAHAEPLKQRMIKIRCM

GFADV    561 KTINPTTKITPGMLKKWLR------------------ETTGANYPVTSAEKNTGFLQPSTAESAGGANTTKSDTQRKVVD
M20036   549 KTINPKTKITPGMLKRWLNTWDRQPIQLSHEMPELYLETTGPNSSATTATKNTGNSQPTTAKSAESVNTENCDTPKRSAS
X77084   549 KPVNPKTKVTPGMLKTWISTWDRIPIKLSHEMPELYLETSGPNSSATTATKSTGNLQPTTAETAESVNTANCDTPKRGAS
X77085   549 KTINPKTKITPGMLKRWLNTWDRQPIQLSHEMPELYLETTGPNSSATSATKTTGNSQPTTAKTAESVSTADCDTPKRGAS

GFADV    623 SAPLKAAKRPRHE
M20036   629 SVPAKQHKRPRHE
X77084   629 SVPPKQHKRPRHE
X77085   629 SVPPKQHKRPRHE
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Technical Appendix Figure 4. Nonstructural protein 1 alignment of gray fox amdovirus and representative amdoviruses. Black highlights 70%–100% similarity, gray highlights 50–70% similarity.

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GFADV       1 MDSNETEQMDISEQQ-QVAEHGG-------------GVGKSTGGFNNTTEFKVTNNEVIITCNATRVVHINQASTDEYLI
M20036      1 MDSTEAEQMDTEQATNQTAEAGGGGGGGGGGGGGGGGVGNSTGGFNNTTEFKVINNEVYITCHATRMVHINQADTDEYLI
GU183265    1 MDSTEPEQMDTEQATNQTAEAGGGGGG-GGGGGGGGGVGNSTGGFNNTTEFKVINNEVYITCHATRMVHINQADTDEYLI
DQ371395    1 MDSTEPEQMDTEQATNQTAEAGGGGGGSGGGGGGGGGVGNSTGGFNNTTEFKVINNEVYITCHATRMVHINQADTDEYLI

GFADV      67 FNAGREID-KTPKGSLNLEFFMFDDFHQQVMTPWFLVDSNAWGVWMSPKDFQQMKVLCSEIELVSLEQEIDNVVIKTVTE
M20036     81 FNAGRTTDTKTHQQKLNLEFFVYDDFHQQVMTPWYIVDSNAWGVWMSPKDFQQMKTLCSEISLVTLEQEIDNVTIKTVTE
GU183265   80 FNAGRTTDTKTAQKKLDLEFFVYDDFHQQVMTPWFLVDSNAWGVWMSPKDFQQMKTLCSEISLVTLEQEIDNVTIKTVTE
DQ371395   81 FNAGRTTDTKTAQKKLNLEFFVYDDFHQQVMTPWFLVDSNAWGVWMSPKDFQQMKTLCSDISLISLEQEIDNVTIKTVTE

GFADV     146 TTQCGTSVIQYNNDLTASLQVALDTNNILPYTPAAPLQETLGFVPWRATKPTQYRYYHPSYIYNRYPNIQKKSQEQSTYT
M20036    161 TNQGNASTKQFNNDLTASLQVALDTNNILPYTPAAPLGETLGFVPWRATKPTQYRYYHPCYIYNRYPNIQKVATETLTWD
GU183265  160 TNQGNASTKQFNNDLTASLQVALDTNNILPYTPAAPLGETLGFIPWRATKPTQYRYYHPCYIYNRYPNIQKTGQQQLEWT
DQ371395  161 TNQGNASTKQFNNDLTASLQVALDTNNILPYTPAAPLGETLGFVPWRATKPTQYRYYHPCYIYNRYPNIQKTG-QELEWT

GFADV     226 AIQDDYFSVDQDYFNFITIENNIPIEILRTGDNFHTGRYEFKSKPCKLTMSYQSTRCLGLPPLCKPKTD-TKLVTSLENG
M20036    241 AVQDDYLSVDEQYFNFITIENNIPINILRTGDNFHTGLYEFNSKPCKLTLSYQSTRCLGLPPLCKPKTDTTHKVTSKENG
GU183265  240 GTQDDYLSVDEQYFNFITVENNIPINILRTGDNFHTGLYEFKSKPCKLTLSYQSTRCLGLPPLCKPKTDVTHKVTSLQNG
DQ371395  240 GTQDDYLSVDEQYFNFITIENNIPINILRTGDNFHTGLYEFKSKPCKLTFSYQSTRCLGLPPLCKPKTDKTHKATSKENG

GFADV     305 GEMDCYVEGQQHTRKGHFWGEEKAEKNTEINRVRPYNIGYQFPEWIVPAGLQGSYFTGGPRQWSDTTKGAGTHSQTVQQN
M20036    321 ADL-IYIQGQDNTRLGHFWGEERGKKNAEMNRIRPYNIGYQYPEWIIPAGLQGSYFAGGPRQWSDTTKGAGTHSQHLQQN
GU183265  320 ADL-IYIEGQDNTRLGHFWGEERGKKNAEMNRVRPYNIGFQYPEWIIPAGLQGSYFAGGPRQWSDTTKGAGTHSQQLQQN
DQ371395  320 ADL-IDVQRQRNTRLGHFWGEQRGKKNAEMNRIRPYNIGNQYPEWIIPAGLKGSYFAGGQRQWSDTTKGAGTHSQNLKQN

GFADV     385 FSTRYVFDRNHGGDNQVDTIDSIPIQERGKYYSEQELQQATTLTATNKAQ--TGKKWESWEEEGWPAASGTHFEDEVLYF
M20036    400 FSTRYIYDRNHGGDNEVDLLDGIPIHERSNYYSDNEIEQHTAKQPKLRTPPIHHSKIDSWEEEGWPAASGTHFEDEVIYL
GU183265  399 FSTRYIYDRNHGGDNEVDLLDGIPIHERSNYYSDHEIEQHTAKQPKLRVPPIHHSKIDSWEEEGWPAASGTHFEDEVIYL
DQ371395  399 FSTRYIYDRNNGGDKEVNLLDGIPIHERSKYYSDNEIEQHTAKQPKLRTPPIHHSKIDSWEEEGWPAASGTHFEDEVIYL

GFADV     463 DYFNFSGETTKNFPQEVLSDGLQMKKLLNAYQPTVGQDDVGPVYPWGQIWDKKPDMDHKPSMNYNAPFVCKNNPPGQLLV
M20036    480 DYFNFSGEQELNFPHEVLDDAAQMKKLLNSYQPTVAQDNVGPVYPWGQIWDKKPHMDHKPSMNNNAPFVCKNNPPGQLFV
GU183265  479 DYFNFGGEQTLEFPHEVLDDAAQMKKLLNSYQPTVAQDNVGPVYPWGQIWDKKPDMDHKPSMNNNAPFVCKNNPPGQLFV
DQ371395  479 DYFNLSGETELEFPHEVLDDAAQMKKLLNSYQPTVALDNVGPVYPWGQIWDKKPDMDHKPSMNNSASFVCKNNPPGQLFV

GFADV     543 KLTENLTDKFNYDEDPDRIKTYGYFTWRGKLTLRGKLGQTSCWNPVKRERIGEPGVFSKSNYHKQIPNNRGNFEIGIQYG
M20036    560 KLTENLTDTFNYDENPDRIKTYGYFTWRGKLVLKGKLSQVTCWNPVKRELIGEPGVFTKDKYHKQIPNNKGNFEIGLQYG
GU183265  559 KLTENLTDTFNYDENPDRIKTYGYFTWRGKLVLKGKLSQVTCWNPVKRELIGEPGVFTKDNYHKQIPNNKGNFEIGLQYG
DQ371395  559 KLTENLTDTFNYDENPDRIKTYGYFTWRGKLVTKGKLSQVTCWSPVKRELIGEPGVFTKDNYHKQIPNNKGNYEIGLQYG

GFADV     623 RSTVKYLY
M20036    640 RSTIKYIY
GU183265  639 RSTIKYIY
DQ371395  639 RSTIKYIY
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Technical Appendix Figure 5. Viral protein 2 alignment of gray fox amdovirus and representative amdoviruses. Black highlights 70%–100% similarity, gray highlights 50–70% similarity.