

1 Genetic diversity of species *Fowl aviadenovirus D* and *Fowl aviadenovirus E*

2 Short title: Genome sequences of FAdV-D and FAdV-E members

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22 Word count of abstract: 134

23 Word count of main text: 2,800

24 The GenBank accession numbers for the genome sequences of 685, SR48, SR49,
25 CR119, YR36, TR59, 764 and 380 are KT862805 to KT862812, respectively

26 **Keywords**

27 *Aviadenovirus*, *Fowl aviadenovirus D*, *Fowl aviadenovirus E*, whole genome sequence,
28 phylogeny

29 **Abbreviations**

30 AGE-adenoviral gizzard erosions, DAdV-B-*Duck aviadenovirus B*, FAdV-A to FAdV-E-
31 *Fowl aviadenovirus A* to *Fowl aviadenovirus E*, GoAdV-A-*Goose aviadenovirus A*, HAdVs-
32 human adenoviruses, HHS-hepatitis-hydropericardium syndrome, IBH-inclusion body
33 hepatitis, PiAdV-A-*Pigeon aviadenovirus A*, PsAdV-psittacine adenovirus, RAdV-raptor
34 adenovirus, SPSkAdV-South Polar skua adenovirus, TAdV-B to TAdV-D-*Turkey*
35 *aviadenovirus B* to *Turkey aviadenovirus D*

36

37 **Abstract**

38 Complete genomes of eight reference strains representing different serotypes within
39 species *Fowl aviadenovirus D* (FAdV-D) and *Fowl aviadenovirus E* (FAdV-E) were
40 sequenced. The sequenced genomes of FAdV-D and FAdV-E members comprise 43,287 to
41 44,336 bp, and have a gene organization identical to that of an earlier sequenced FAdV-D
42 member (strain A-2A). Highest diversity was noticed in the hexon and fiber genes and
43 ORF19. All genomes, sequenced in this study, contain one fiber gene. Phylogenetic analyses
44 and G+C content support the division of the genus *Aviadenovirus* into the currently
45 recognized species. Our data also suggest that the strain SR48 should be considered as FAdV-
46 11 instead of FAdV-2 and similarly the strain HG as FAdV-8b. The present results complete
47 the list of genome sequences of reference strains representing all serotypes in species FAdV-
48 D and FAdV-E.

49

50 **Introduction**

51 Aviadenoviruses infect avian hosts exclusively. Fowl aviadenoviruses (FAdVs) are
52 grouped into five species (*Fowl aviadenovirus A* to *Fowl aviadenovirus E*) in the genus
53 *Aviadenovirus* based on genome organization and phylogeny (Harrach et al., 2011; Harrach &
54 Kajan, 2011). An informal abbreviation of FAdV species such as for example FAdV-A for
55 *Fowl aviadenovirus A* will be used in the following part of this paper. FAdVs are widely
56 distributed, and some of them cause inclusion body hepatitis (IBH), hepatitis-
57 hydropericardium syndrome (HHS) and adenoviral gizzard erosions (AGE) in chickens (Hess,
58 2013). FAdV strains belonging to species FAdV-D and FAdV-E have been isolated mostly
59 from IBH cases and members of species FAdV-C from HHS outbreaks (Hess et al., 1999;
60 Ojkic et al., 2008; Slavec et al., 2013; Steer et al., 2011; Zadavec et al., 2011). AGE,
61 associated with FAdV-1 infection, have been described in chickens in Japan and Europe
62 (Domanska-Blicharz et al., 2011; Kecskeméti et al., 2012; Manarolla et al., 2009; Marek et
63 al., 2010a; Ono et al., 2001). Before the era of DNA sequencing, serology was the principal
64 means of identifying aviadenovirus types and the 12 serotypes have been grouped into five
65 FAdV species recognized to date as follows: FAdV-A (FAdV-1), FAdV-B (FAdV-5), FAdV-
66 C (FAdV-4 and FAdV-10), FAdV-D (FAdV-2, FAdV-3, FAdV-9 and FAdV-11) and FAdV-
67 E (FAdV-6, FAdV-7, FAdV-8a and FAdV-8b) (Harrach et al., 2011; Hess, 2000). DNA
68 sequencing of the loop 1 (L1) region of the hexon gene is now used frequently for typing
69 FAdVs (Kajan et al., 2013; Marek et al., 2010b; Meulemanns et al., 2004; Raue & Hess,
70 1998).

71 High-throughput sequencing became popular in recent years since it permits the rapid
72 and comprehensive analysis of complete aviadenovirus genomes. At least one complete
73 genome sequence is available now for all FAdV species, including: FAdV-A (FAdV-1, also
74 known as CELO virus), FAdV-B (FAdV-5 strain 340), FAdV-C (FAdV-4 strains ON1 and
75 KR5), FAdV-D (FAdV-9 strain A-2A) and FAdV-E (FAdV-8 strain HG) (Chiocca et al.,

76 1996; Grgic et al., 2011; Griffin & Nagy, 2011; Marek et al., 2012, 2013; Ojkic & Nagy,
77 2000). In addition, the whole genome of numerous non-chicken aviadenoviruses have also
78 been sequenced. They are TAdV-1 (*Turkey aviadenovirus B*, TAdV-B), GoAdV-4 (*Goose*
79 *aviadenovirus A*, GoAdV-A), TAdV-4 (*Turkey aviadenovirus C*, TAdV-C), TAdV-5 (*Turkey*
80 *aviadenovirus D*, TAdV-D), PiAdV-1 (*Pigeon aviadenovirus A*, PiAdV-A) and DAdV-2
81 (*Duck aviadenovirus B*, DAdV-B) (Kajan et al., 2010, 2012; Marek et al., 2014a, 2014b).

82 Adenoviruses in general are thought to have co-evolved with a wide range of
83 vertebrate hosts, and thus the genus *Aviadenovirus* with the birds (Harrach, 2014). In this
84 genus, we can indeed observe at least two major clusters containing the AdVs of the
85 anseriform birds (DAdV-B and GoAdV-A), and the other the AdVs originating from the
86 galliformes, i.e. turkey and fowl adenoviruses (Marek et al., 2014b). The two species that
87 seem to contain the majority of FAdV sero- and genotypes are FAdV-D and -E encompassing
88 eight different FAdV types (Marek et al., 2010b). This might indicate that the viruses in these
89 species have been coevolving with chickens for a long period. However, the close relatedness
90 and mixed phylogenetic position of the turkey and fowl adenoviruses within the galliform
91 AdV clade (Marek et al. 2014a), as well as the high pathogenicity of certain FAdV types
92 imply that host switches also might have occurred. The increased pathogenicity of a virus is
93 often the consequence of a host switch (Benko & Harrach, 2003; Kohl et al., 2012).

94 For the correct reconstruction of the aviadenovirus evolution, it is important to analyze
95 the whole genomes of additional isolates, first of all strains representing yet not examined
96 FAdV types. The main purpose of this study was to obtain the complete genome sequences of
97 reference strains of different types belonging to species FAdV-D and FAdV-E by high-
98 throughput sequencing technology. With the completion of these genome sequences, we
99 expected to gain additional insights into the evolution of the genus *Aviadenovirus*.

100 **Results**

101 Genome organization

102 After filtering for contaminating chicken chromosomal sequence reads, the average
103 coverage for all sequenced genomes was between 250 and 27,000 reads per nucleotide. *De*
104 *novo* assembly was optimal when using 1 to 100% of these data (depending on the coverage).
105 Gap closure by PCR and Sanger sequencing resulted in final genome sequences ranging
106 between 43,287 and 44,336 bp with nucleotide composition ranging between 52.8 and 58.0%
107 G+C content (Table 1). The percentage sequence identities to available complete
108 aviadenovirus genome sequences are summarized in Table 2. The intraspecies sequence
109 identities varied between 89.4 and 97.1% for different FAdV-D strains and 92.7 and 97.1%
110 for different FAdV-E strains. The interspecies nucleotide sequence identities varied between
111 71.2 and 75.4% for FAdV-D and FAdV-E strains. Strain SR48 (FAdV-2) showed higher
112 sequence identity to strain 380 (FAdV-11, 97.1%) than to strain 685 (FAdV-2, 95.8%). Strain
113 HG (FAdV-8) showed higher sequence identity to strain 764 (FAdV-8b, 97.1%) than to strain
114 TR59 (FAdV-8a, 94.1%). Interestingly, strain CR119 (FAdV-6) shared very high sequence
115 identity (97.0%) with strain YR36 (FAdV-7).

116 All sequenced genomes had a gene organization identical to that of the previously
117 sequenced FAdV-9 (FAdV-D strain A-2A) (Fig. 1).

118 Global pairwise sequence alignment analyses identified areas of great interspecies
119 diversities. The results for one member of the FAdV-D and FAdV-E species (685 and CR119,
120 respectively) are shown in Fig.1 and for all other FAdV-D and FAdV-E members in Fig. S1.
121 The genomes of FAdV-D and FAdV-E members display high sequence conservation in the
122 central genomic region (from IVa2 to fiber gene) with all aviadenovirus genomes, and in the
123 terminal genomic regions with each other as well. The terminal regions show lower sequence
124 conservation or none with other aviadenoviruses.

125 All FAdV-D members sequenced until now show high sequence conservation
126 throughout the genome. However, strain 685 has an additional non-coding sequence region

127 near the right genome end in comparison to other sequenced FAdV-D strains (Fig. 1). Strain
128 SR49 shows lower sequence conservation with other sequenced FAdV-D strains in the region
129 from approximately 20 kb to 37 kb (Fig. S1). Strain A-2A showed lower sequence
130 conservation with most FAdV-D strains within hexon and fiber genes and has an additional
131 sequence region near the right genome end in comparison to other sequenced FAdV-D strains.
132 Strains HBQ12 and BJH13 also have an additional sequence region near the right genome end
133 in comparison to all FAdV-D strains sequenced in this study.

134 The hexon, fiber, and ORF19 are among the most variable genes among the FAdV-E
135 members (Fig. 1 and Fig. S1). Hexon shows lower sequence conservation in all FAdV-E
136 strains (only strains 764 and HG have similar hexon genes). Strains CR119 and TR59 show
137 lower sequence conservation within fiber gene as compared to other FAdV-E strains, whilst
138 strains YR36, 764 and HG possess similar fiber genes. ORF19 was similar in strains CR119,
139 YR36 and TR59, but different to that of strains 764 and HG. Sequence region near the right
140 genome end was similar in strains CR119 and YR36, but different from that of strains TR59,
141 764 and HG. In addition, strain HG shows lower sequence conservation with FAdV-E strains
142 sequenced in this study within the pTP gene.

143 Phylogeny

144 Phylogenetic analyses of the whole genomes (Fig. 2) or selected proteins (Fig. 3) of
145 various AdVs supported the division of the genus *Aviadenovirus* into the currently recognized
146 species. Strains SR48 and 380, and also strains HG and 764 are monophyletic in the whole
147 genome and in the hexon analysis, too (Fig. 2, Fig. 3).

148 Discussion

149 The genus *Aviadenovirus* encompasses fowl aviadenoviruses (FAdVs), which were
150 grouped into 12 serotypes (FAdV-1 to -8a and -8b to -11) based on cross-neutralization tests
151 (Hess, 2000). Recently, at least 12 genotypes were revealed by sequence analysis of the hexon

152 loop 1 (L1) region (Marek et al., 2010b). The 12 serotypes constitute five “groups” (now
153 species *Fowl aviadenovirus A* to *Fowl aviadenovirus E*) initially established on the basis of
154 restriction enzyme digest pattern of whole genomes (Zsak & Kisary, 1984). Phylogenetic and
155 sequence analyses of whole genomes supported the division of the genus *Aviadenovirus* into
156 the currently recognized species (Marek et al., 2012, 2013, 2014a, 2014b, Pauly et al., 2015).

157 Whole genome sequence identities among members of the various officially accepted
158 aviadenovirus species range from 42.4% (between TAdV-1 (TAdV-B) and GoAdV-4
159 (GoAdV-A)) to 72.2% (between FAdV-9 (FAdV-D) and FAdV-8b (FAdV-E)) (Marek et al.,
160 2013). In the present study, phylogenetic and sequence analyses confirmed the present
161 division of the genus *Aviadenovirus* into species. The lowest genome sequence identity
162 between the FAdV-D and FAdV-E members and members of different aviadenovirus species
163 was 45.9% (between the FAdV-2 (strain 685) and DAdV-2 (DAdV-B)) and 45.0% (between
164 the FAdV-8b (strain HG) and DAdV-2 (DAdV-B)), respectively. The highest genome
165 sequence identity was 75.4% (between FAdV-8b (FAdV-E strain 764) and FAdV-3 (FAdV-D
166 strain SR49)) (Table 2). Phylogenetic analysis based on the amino acid sequence of the DNA
167 polymerases show phylogenetic differences greater than the required 5-15% (Fig. 3b).
168 Therefore, although FAdV-D and FAdV-E are closely genetically related (Grgic et al., 2011;
169 Marek et al., 2010b, 2012, 2013, 2014a, 2014b), they represent two different aviadenovirus
170 species which is also supported by differences in the G+C content (Table 1).

171 Up to now, the complete genome sequence for a member of FAdV-E was only
172 available for the isolate HG (Grgic et al., 2011). This strain was labeled as FAdV-8 and was
173 so far not assigned to a FAdV type (FAdV-8a or -8b). However, based on partial hexon gene
174 sequences, the clustering of this strain together with FAdV-8b strains was already observed
175 (Marek et al., 2014a). This is now supported by the full genome sequence. Originally, typing
176 of FAdV was achieved by cross-neutralization test and the strain SR48 was considered as a
177 reference strain of FAdV-2 (McFerran & Connor, 1977). However, partial hexon gene

178 sequences demonstrated the grouping of this strain together with FAdV-11 strains (Marek et
179 al., 2010b; Meulemanns et al., 2004). The present study confirms the grouping of strain SR48
180 within FAdV-11, based on adequate phylogenetic and genome sequence similarities, which
181 should be considered in future studies (Table 2, Fig. 2, Fig. 3). This re-assignment is also
182 supported by recently published neutralization assay in which SR48 was used as reference
183 strain (Steer et al., 2011).

184 Genes, inherited by all modern AdVs from their common ancestor, are located
185 centrally in the genome and additional, niche specific genes, have accumulated in each
186 lineage, mostly near the genome termini (Davison et al., 2003). In this study, it was shown
187 that the terminal regions of the genome have the most variable sequences in members of
188 aviadenovirus species as well (Fig. 1, Fig. S1). However, it is still not clear which genetic
189 features enable a virus to cause specific disease. Recently, the genomic conservation and
190 diversity among human adenoviruses (HAdVs) were examined and the penton base, hexon
191 and fiber ORFs and E3 regions were shown to be among the most variable in the HAdV-D
192 genomes (Robinson et al., 2011). As their protein products mediate uptake of the virus into
193 the target cell and/or host immune system recognition of the virus, they may be targets for
194 selective evolutionary pressure. In the present study, hexon, fiber and ORF19 were shown to
195 be among the most variable in the FAdV-D and FAdV-E genomes (Fig. S1). For HAdVs, the
196 areas of greatest intraspecies diversity were different for different species. In this study, the
197 same phenomenon could be observed even between strains belonging to different types within
198 the same species. It would be interesting to further analyze the whole genomes of additional
199 isolates belonging to different aviadenovirus species.

200 Viruses co-evolving for long time with their host are thought to be well adapted and
201 not markedly pathogenic. We suggested earlier that viruses in species FAdV-D and FAdV-E
202 have been coevolving with chickens for a long period (Marek et al., 2014b). However, FAdVs
203 most commonly isolated from IBH cases in chickens belong to FAdV-D and FAdV-E (Kajan

204 et al., 2013; Marek et al., 2010b; Ojkic et al., 2008). Beach *et al.* (2009) noticed genetic
205 differences between virulent and non-virulent turkey haemorrhagic enteritis virus isolates (a
206 member of the genus *Siadenovirus*) within ORF1, E3 and the fiber protein. However, Grgic *et*
207 *al.* (2014) did not notice significant differences between fibers of virulent and apathogenic
208 FAdV isolates, which was recently confirmed by Schachner *et al.* (2016). In order to estimate
209 the influence of viral genetics on pathology, experimental infections with different
210 molecularly manipulated isolates would be necessary.

211 **Conclusion**

212 The complete genome sequence of FAdV reference strains 685, SR48, SR49, 380,
213 CR119, YR36, TR59 and 764 were obtained by Illumina sequencing. Phylogenetic and
214 sequence analyses of the whole genomes support the division of the genus *Aviadenovirus* into
215 the currently recognized species. The sequenced genomes of FAdV-D and FAdV-E members
216 have a genome organization identical to that of earlier sequenced FAdV-D member (strain A-
217 2A). The data suggest a common evolutionary origin of strains SR48 and 380, and also of
218 strains HG and 764. Complete genome sequence information of aviadenoviruses is important
219 for taxonomy, diagnostics and pathogenicity studies.

220 **Materials and methods**

221 Virus isolates

222 Eight reference FAdV strains (Kawamura et al., 1964, McFerran et al., 1972)
223 representing different types within the species FAdV-D and FAdV-E (Table 1) were
224 propagated, after plaque purification, on confluent monolayers of chicken embryo liver cells
225 as described previously (Marek et al., 2010b).

226 DNA extraction

227 Cell culture supernatants were clarified by low speed centrifugation (10 min at 2,000
228 g) and then ultracentrifuged (3 h at 140,000 g). The pelleted cell-free virions were used for

229 DNA isolation (Marek et al., 2012). The presence of virus DNA in the sample was verified by
230 PCR targeting the hexon gene (HexA/HexB) (Meulemans et al., 2004).

231 Illumina sequencing

232 Whole genome sequencing was performed by using an Illumina system (HiSeq2000,
233 BGI, Hong Kong for 685 and GAIIX, Central Service Facility NGS Unit, Vienna, Austria for
234 SR48, SR49, 380, CR119, YR36, TR59 and 764). Paired-end libraries were generated.
235 Multiple virus samples were sequenced in a single lane and sequence reads corresponding to
236 the individual strains were separated by barcoding. Due to propagation of the strains in
237 chicken cells, contamination by chicken genome reads was anticipated. Therefore, all reads
238 were mapped initially against the available genome of *Gallus gallus* (v. 3.0) and the
239 mitochondrial genome of *Gallus sonneratii* (AP006746.1), and only the unmapped reads were
240 used for assembly of the viral genomes (Marek et al., 2012).

241 *De novo* assembly

242 Excess coverage might hamper *de novo* assembly. Therefore, we sub-sampled
243 different numbers of reads for different strains (Marek et al., 2013). The whole genome
244 sequences were then assembled by using the CLC Genomics Workbench v. 4.0 (CLC bio,
245 Aarhus, Denmark). By comparison with sequences available for various complete
246 aviadenovirus genomes and for the left and right ends of several additional FAdV genomes
247 (Corredor et al., 2008; Corredor et al., 2006), the resulting contigs were manually ordered and
248 orientated (Marek et al., 2012). The contig sequences were aligned by using the Accelrys
249 Gene version 2.5 (Accelrys, San Diego, CA).

250 Gap closure using PCR and Sanger sequencing

251 In order to close the gaps between contigs by Sanger sequencing, PCR primers were
252 designed on the basis of the sequences at contig ends. Oligonucleotide primers for amplifying
253 the sequences at one genome end were designed based on obtained sequences from the other

254 genome end because of the symmetric nature of the inverted terminal repeat. Primer
255 sequences are available from the authors upon request. Sanger sequencing services were
256 provided by the LGC Genomics (Berlin, Germany). The complete genome sequences for
257 strains 685, SR48, SR49, CR119, YR36, TR59, 764 and 380 were submitted to the GenBank
258 database and assigned to accession numbers KT862805 to KT862812, respectively (Table 1).

259 Annotation and phylogenetic analyses

260 FAdV genomes were annotated as described earlier (Marek et al., 2014b). Percentage
261 sequence identities of whole aviadenovirus genome sequences were calculated using the
262 Lasergene software (DNASTAR Inc., Madison, WI). Three phylogenetic calculations were
263 performed to assess the correct relationship of the examined strains: based on the complete
264 genome, the amino acid sequence of the viral DNA polymerase, and the amino acid sequence
265 of the hexon, the major capsid protein. The genomes were aligned using PRANK (Löytynoja
266 & Goldman, 2010), while the protein sequences were aligned using MAFFT and the
267 alignments were edited manually using BioEdit (Hall 1999; Katoh & Toh, 2008). The edited
268 alignment lengths were 106,920 nt, 1020 aa and 896 aa for the complete genome, DNA
269 polymerase and hexon alignments, respectively. The best evolutionary model was GTR+ Γ for
270 the tree inference of complete genomes, and it was predicted using ProtTest (Darriba et al.,
271 2011) for the protein sequences (DNA polymerase: LG+I+ Γ , hexon: LG+ Γ +F). Phylogenetic
272 analyses were performed using maximum likelihood methods within the RAxML software
273 package (Stamatakis, 2014). Clade support was assessed by using non-parametric
274 bootstrapping with 1000 replicates, the sequenced strains were compared to all published
275 genome sequences of avian AdVs. Global pairwise alignments to assess sequence identities
276 were performed using mVISTA LAGAN (Brudno et al., 2003).

277 Acknowledgements

278 The authors thank Irina Prokofieva and Evelyn Berger for their excellent technical
279 help. The genome analysis work was supported partially by the Hungarian Research Fund
280 grant K100163.

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439

440 **Figure legends**

441 **Figure 1.** Global comparisons of the genome sequences of (a) FAdV-2 (FAdV-D strain 685)
442 and (b) FAdV-6 (FAdV-E strain CR119) with those of other aviadenoviruses. Peaks show
443 regions having >50% sequence identity. At the top, the rightward- and leftward-transcribed
444 strands of the genome are shown in grey with indicated a 2,000-nucleotide scale on the latter
445 one. The six reading frames are shown in light grey above and below the genome. Protein-
446 encoding regions are depicted as colored arrows and bars (the ORF prefix omitted). The genes
447 marked by red arrows are conserved in every AdV sequenced to date. Those colored green
448 have orthologues in other aviadenoviruses only. Splice sites are indicated by diagonal lines.
449 DBP, DNA-binding protein; ITR, inverted terminal repeat (colored blue); pTP, terminal
450 protein precursor; * proposed FAdV-11

451 **Figure 2.** Phylogenetic tree based on all available whole genome sequences of avian AdVs.
452 Genomes of strains 685, SR48, SR49, 380, CR119, YR36, TR59 and 764 (printed in bold)
453 were sequenced in this study whereas the other avian AdV genome sequences have been
454 published previously (Chioccia et al., 1996; Grgic et al., 2011; Griffin and Nagy, 2011; Hess et
455 al., 1997; Kajan et al., 2010, 2012; Kovacs and Benko, 2011; Marek et al., 2012, 2013, 2014;
456 Ojkic and Nagy, 2000; Park et al., 2012; Pitcovski et al., 1998; To et al., 2014; Vera-
457 Hernández et al., 2015; Zhao et al., 2015). Branch lengths are given in number of
458 substitutions per site (see the scale). Bootstrap values are given in percentage for 1000
459 datasets, the tree was rooted at the midpoint. * Proposed FAdV-11. AdV, adenovirus; DAdV,
460 duck AdV; FAdV, fowl AdV; GoAdV, goose AdV; PiAdV, pigeon AdV; PsAdV, psittacine
461 AdV; RAdV, raptor AdV; SPSkAdV, South Polar skua AdV; SkAdV-A, *Skua siadenovirus*
462 *A*; TAdV, turkey AdV.

463 **Figure 3.** Phylogenetic trees based on derived amino acid sequences of adenoviral DNA
464 polymerase (A) and hexon (B) sequences. The inset in (B) shows the close-up of species *Fowl*

465 *aviadenovirus D* and *Fowl aviadenovirus E*. Branch lengths are given in number of
466 substitutions per site (see the scale). Bootstrap values are given in percentage for 1000
467 datasets if they exceeded 75%. The viruses, sequenced in this study, are printed in bold. The
468 trees were rooted at the midpoint. * proposed FAdV-11. ** The fowl aviadenovirus 9 DNA
469 polymerase amino acid sequence was derived from the given NCBI Nucleotide sequence.
470 AdV, adenovirus; DAdV, duck AdV; FaAdV, falcon AdV; FAdV, fowl AdV; GoAdV, goose
471 AdV; GTAdV, great tit AdV; GuAdV, gull AdV; PiAdV, pigeon AdV; PsAdV, psittacine
472 AdV; RAdV, raptor AdV; SPSkAdV, South Polar skua AdV; SkAdV-A, *Skua siadenovirus*
473 *A*; TAdV, turkey AdV.

474 **Figure S1.** Global comparisons of the genome sequences of FAdV-D and FAdV-E members
475 with those of other aviadenoviruses. Peaks show regions sharing sequence identity higher than
476 50%. * proposed FAdV-11.

477

478	Virus strain	genome length (bp)	G+C%	accession number	species/type
479	685	44,336	53.3	KT862805	FAdV-D/FAdV-2
480	SR48	43,632	53.3	KT862806	FAdV-D/FAdV-2*
481	SR49	43,337	52.8	KT862807	FAdV-D/FAdV-3
482	380	43,347	53.3	KT862812	FAdV-D/FAdV-11
483	CR119	43,810	57.8	KT862808	FAdV-E/FAdV-6
484	YR36	43,525	57.8	KT862809	FAdV-E/FAdV-7
485	TR59	43,287	58.0	KT862810	FAdV-E/FAdV-8a
486	764	43,666	57.8	KT862811	FAdV-E/FAdV-8b

487 * proposed FAdV-11

488 Table 1. List of isolates used in this study.

	685 FAdV-2 FAdV-D	SR48 FAdV-2* FAdV-D	SR49 FAdV-3 FAdV-D	HBQ12 FAdV-D	BJH13 FAdV-D	380 FAdV-11 FAdV-D	A-2A FAdV-9 FAdV-D	CR119 FAdV-6 FAdV-E	YR36 FAdV-7 FAdV-E	TR59 FAdV-8a FAdV-E	764 FAdV-8b FAdV-E	HG FAdV-8b FAdV-E	CELO FAdV-1 FAdV-A	340 FAdV-5 FAdV-B	KR5 FAdV-4 FAdV-C	ONI FAdV-4 FAdV-C	JSJ13 FAdV-C	MX-SHP95 FAdV-C	D90/2 TAdV-1 TAdV-B	TNI1 TAdV-4 TAdV-C	1277BT TAdV-5 TAdV-D	IDA4 PiAdV-1 PiAdV-A	GR DAdV-2 DAdV-B	P29 GoAdV-4 GoAdV-A
685	100	95.8	89.5	95.6	95.5	95.5	91.3	74.2	74.1	74.2	74.5	73.8	52.7	63.3	57.5	57.6	57.0	57.8	51.5	64.7	52.6	48.0	45.9	46.0
SR48		100	90.8	96.8	96.5	97.1	93.4	74.6	74.5	74.3	74.9	74.2	53.1	64.2	57.9	57.9	57.3	57.9	51.7	64.8	52.8	48.1	46.0	46.5
SR49			100	90.2	90.3	90.7	89.4	75.0	75.0	74.7	75.4	74.5	53.0	63.5	57.6	57.7	56.9	57.6	51.5	64.1	52.8	48.0	46.0	46.3
HBQ12				100	99.8	95.8	94.5	74.0	73.9	73.7	74.4	73.7	53.2	64.3	58.1	58.0	57.5	58.1	51.7	64.7	52.8	48.2	46.2	46.5
BJH13					100	96.0	94.2	74.1	74.0	73.9	74.5	73.5	53.2	64.3	58.0	58.0	57.3	58.0	51.9	64.5	53.0	48.4	46.2	46.5
380						100	92.4	74.7	74.5	74.6	75.0	74.0	52.8	63.7	57.6	57.7	56.9	57.6	51.5	64.4	52.6	48.1	45.9	46.2
A-2A							100	71.5	71.4	71.5	71.9	71.2	51.6	62.6	56.5	56.4	55.9	56.5	49.9	63.0	51.2	46.4	46.4	46.9
CR119								100	97.0	94.0	93.5	92.7	52.7	63.9	57.5	57.5	56.8	57.5	53.0	63.7	52.1	48.8	45.4	45.6
YR36									100	93.9	94.0	93.1	52.9	64.0	57.7	57.7	56.9	57.7	53.0	63.9	52.3	48.9	45.6	45.6
TR59										100	94.8	94.1	52.6	63.8	57.3	57.3	56.5	57.3	52.9	63.7	52.0	48.6	45.2	45.3
764											100	97.1	53.1	64.2	57.8	57.8	57.0	57.8	53.1	64.1	52.4	49.0	45.6	45.6
HG												100	52.4	63.5	57.2	57.2	56.4	57.2	52.3	63.5	51.6	48.2	45.0	45.0

489 * proposed FAdV-11

490 Table 2. Percentage sequence identities of complete aviadenovirus genomes.