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1 **Full genome sequence of a novel circo-like virus detected in an adult European eel**
2 **(*Anguilla anguilla*) showing signs of cauliflower disease**
3

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25

26 **Abstract**

27 An adult European eel *(Anguilla anguilla)*, showing typical signs of the so-called
28 cauliflower disease, was subjected to pathological and molecular virological examinations.
29 Samples, taken from the internal organs and the polypoid proliferative tissue from the mouth,
30 were examined by PCR for the detection of several viruses. Positive results were obtained
31 with a nested PCR targeting the *rep* gene of circoviruses. Analysis of the partial *rep* sequence
32 indicated the presence of a putative novel circovirus, but attempts to isolate it remained
33 unsuccessful. The missing part of the genome was acquired by an inverse nested PCR with
34 two specific primer pairs, designed from the newly determined *rep* sequence, then genome
35 walking was applied. The circular full genome was found to consist of 1378 nt. Two
36 oppositely oriented ORFs were present. One of them could be identified as a circoviral *rep*
37 gene unambiguously. However, the predicted product of the other ORF, though it is a clear
38 positional counterpart of the *cap* genes, showed no obvious homology to any known
39 circoviral capsid proteins. A stem-loop-like element in the intergenic region between the 5'
40 ends of the ORFs was also found. Phylogenetic calculations indicated that the novel virus
41 belongs to the *Circovirus* genus of the *Circoviridae* family. The relative amount of the viral
42 DNA in the organ samples was estimated by quantitative real-time PCR. The results
43 suggested that the examined fish was caught in an active viraemic status, albeit the role of this
44 circovirus in the etiology of the cauliflower diseases could not be ascertained.

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Introduction

The cauliflower disease (stomatopapilloma or orocutaneous papillomatosis) of the European eel *(Anguilla anguilla*) was first described at the beginning of the 20th century (Wolf 1988). Sporadic occurrence of the disease, with no specificity for age and size of the affected fish, has been reported mostly from the tributary regions of northern European rivers (Delves-Broughton et al. 1980; Wolf 1988). The benign epidermal neoplasms usually grow on the head region, but lesions may occur on other parts of the body (Peters 1975). The papillomas consist of fibro-epithelium and are somtimes pigmented. The cause of the tissue proliferation is unknown. Different viruses, resembling birna-, orthomyxo- and rhabdoviruses have been detected in or isolated from eels with cauliflower disease, but experimental reproduction of the clinical signs has not been successful (Nagabayashi & Wolf 1979; Schwanz-Pfitzer et al. 1984; Ahne & Thomsen, 1985; Ahne et al. 1987). Because of the seasonality of the tumor development, the effect of chemical pollutants and water temperature changes were assumed to contribute to the manifestation of the disease (Peters 1975).

The European eel, presently a critically endangered species, were being introduced to Lake Balaton (the largest lake in Central Europe, located in the western part of Hungary) regularly from 1961 to 1991, except in 1985 and between 1989 and 1990. As a result, more than 83 million elvers (glass eel stage, which start to feed) were stocked in Lake Balaton. By the end of the 1980s, the lake had become overpopulated with eel, and there were two massive periods of eel deaths. These deaths were probably due to a nematode worm (*Anguillicoloides crassus)* infection in 1991 and 1995 (Molnár et al. 1991). Thereafter the Hungarian Government prohibited the introduction of additional eels. By now, the eel stock in Lake Balaton is composed of very old individuals, of which even the youngest are more than 23 years old.

75 In this work, the detection and full genome analysis of a novel circo-like virus are
76 described. The virus was found in different organs of an eel from Lake Balaton which
77 exhibited typical signs of the so-called cauliflower disease.

78 Circoviruses (CVs) are small, naked DNA viruses. The icosahedral virions, with a
79 diameter of 12─26 nm, contain a circular single-stranded DNA genome. The genome size of
80 the CVs studied to date was found to range between 1.7-2.3 kb. The viral capsid is composed
81 of one structural protein (capsid protein) (Biagini et al. 2011). Targeted screenings with a
82 sensitive consensus nested PCR (Halami et al. 2008) resulted in the recognition of a large and
83 still sharply increasing number of novel CVs and circo-like viruses in an incredible diversity
84 of specimens including natural and waste waters, fecal samples from different mammals from
85 bats to man, as well as in several invertebrates and on the surface of a variety of raw meat
86 products (Blinkova et al. 2009; Li et al. 2010; Li et al. 2011). Up until a few years ago, only
87 birds and swine had been known as frequent hosts for CVs. In the past several years, the
88 vertebrate host spectrum has widened to include fish and amphibians (Lőrincz et al. 2011 &
89 2012; Tarján et al. 2014). However, in the majority of these cases, the eventual pathological
90 role of the detected CVs is unknown.

91

92 **Material and methods**

93 **Origin of the sample.** During a routine limnological survey, an approximately 50-cm-long
94 adult eel with cauliflower-like growths on both sides of its mouth was caught in Lake Balaton,
95 Hungary on 21st September 2009. After euthanasia, a necropsy was performed. Small pieces
96 from the labial tissue proliferation, the gills, heart, liver, spleen and kidney were collected in
97 duplicate. One part of the samples was fixed in Bouin's fixative, embedded in paraffin,
98 sectioned (at 4─5 µm), stained with hematoxylin and eosin, and viewed by light microscopy

99 according to standard procedures. The other set of samples were frozen, and used for virus
100 isolation and molecular studies.
101 **Virus isolation.** Virus isolation was attempted on the EK-1 cell line (Chen et al. 1982). The
102 pooled organ homogenates were diluted to a 10% (w/v) suspension in an L-15 medium
103 (Gibco) complemented with antibiotics (Penicillin 300 U/ml, Streptomycin 300 μg/ml). The
104 suspension was centrifuged at 2000 × g for 10 min, and the supernatant was transferred into a
105 new tube immediately. Three parallel inoculations (500 μl suspension per flask) were made in
106 25-cm^2 flasks of EK-1 monolayers at 80% confluency. The flasks were incubated at three
107 temperatures (15°C, 19°C, and 22°C) and checked for the appearance of cytopathic effect
108 (CPE) daily.
109 **PCR.** For PCR, the nucleic acid extraction was made from approximately 25 mg tissue as
110 described in detail by Dán et al. (2003). The tissues were homogenized in a 1× TE buffer, and
111 100 μl from each organ suspension was digested with proteinase K (20 mg/ml) in the
112 presence of sarcosyl (10%). After incubation with guanidine-hydrochloride (8 M) for one
113 hour at room temperature, the DNA was precipitated with ethanol. The presence of adenoviral
114 DNA in the organ samples was tested by a sensitive nested PCR with consensus primers
115 targeting the DNA dependent DNA polymerase gene (Wellehan et al. 2004). For the detection
116 of herpes- irido- or poxviral DNA, a broad spectrum PCR method was used (Hanson et al.
117 2006). Demonstration of the circoviral DNA was attempted by a widely used and very
118 efficient consensus nested PCR described by Halami et al. (2008). For the amplification of the
119 missing part of the putative circular genome of the newly detected CV, two specific primer
120 pairs were designed and used in a nested inverse PCR. The primer sequences were as follows:
121 outer forward: 5'-GCG CTT GAG GAT TCT CAT TC-3'; outer reverse: 5'-CAG ATC GTT
122 CCT CTT CCC TT-3'; inner forward: 5'-GAC TTT GGA TGG AAG AAG CC-3'; inner
123 reverse: 5'-CCT TGT TAT GCT GGT CGT TG-3'.

The PCR program consisted of an initial denaturation step of 98°C for 5 min, followed by 45 cycles of 98°C for 30 sec/56°C for 30 sec/72°C for 60 sec and a final elongation cycle of 72°C for 3 min. The reaction mixture consisted of 34 µl distilled water, 10 µl of 5×HF buffer (Phusion, Thermo Scientific), 0.5 µl Phusion enzyme (Phusion, Thermo Scientific), 1 µl (50 µM) of each (forward and reverse) primer, 1.5 µl of dNTP solution of 10 mM concentration, and 2 µl of the target DNA in a final volume of 50 µl. The reactions were performed in a T1 Thermocycler (Biometra). The results of the PCRs were analyzed by electrophoresis in agarose gels.

Quantitative, real-time PCRs for determining the relative amount of the viral DNA in five organs (the labial proliferative tissue, the gills, liver, spleen and kidney) were carried out in an Applied Biosystems® StepOnePlus™ Real-Time PCR System instrument (Life Technologies). Two specific primers, suitable for the amplification of a 100-bp product were designed. The reaction mixture contained 25 µl 2× PrimeSTAR Max Premix (Takara Bio Inc.), 17.5 µl distilled water, 2.5 µl EvaGreen™ Dye (Biotium) 1 µl of each primer (forward: 5'-AGGCAACGACCAGCATAACA-3'; reverse: 5'-AGTCGTCGATGCAGGCCAAG-3') and 3 µl of the target DNA in a final volume of 50 µl. The program consisted of an initial denaturing at 98°C for 5 min, followed by 40 cycles of 98°C for 10 sec, 55°C for 5 sec, and 72°C for 10 sec. The beta-actin gene (with forward 5'- ACCGGTATCGTCATGGACTC-3'; and reverse 5'-CGTCAGGGTCTTCATCAGGT-3' primers) was used as an internal standard. The results were analyzed by the StepOne Software v2.1 (Applied Biosystems).

Sequencing and sequence analyses. All PCR products were excised from the gels, purified with the QIAquick Gel Extraction Kit (Qiagen), and sequenced directly with the inner primers. The sequencing reactions were performed with the use of the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The electrophoresis was carried out by a commercial service provider on an ABI PRISM 3100 Genetic Analyzer. The amplification

149 product from the inverse PCR, encompassing more that 1000 bp, was cloned with the use of
150 the CloneJET Kit (Fermentas), and sequenced with the primers supplied with the kit. The
151 newly obtained nucleotide sequences were compared with their homologs in the GenBank by
152 using different BLAST algorithms at the NCBI portal. The genome was assembled manually
153 and confirmed with the use of the Staden Package as described elsewhere recently (Doszpoly
154 & Shchelkunov 2010). The size and orientation of ORFs and putative genes were examined
155 after a 6-frame translation of the genomic DNA with the use of the JavaScript DNA
156 Translator 1.1 program (Perry 2003).
157 **Phylogeny inference.** Phylogenetic calculations were performed online at the Mobyle portal
158 (http://mobyle.pasteur.fr/cgi-bin/portal.py) of the Pasteur Institute (Paris) using the distance
159 matrix analysis (Protdist) with the Jones–Taylor–Thornton matrix, then the Fitch calculations
160 were performed with global rearrangements. The tree topology was tested by bootstrap
161 analysis (Seqboot/1000 samplings, Protdist, Fitch, Consense).
162

163 **Results**

164 **Gross and Microscopic Pathology.** On both sides of the mouth, soft polypoid, raspberry-
165 coloured masses (approximately 1 cm in diameter) were seen (Fig 1a). The fish was slightly
166 emaciated. The only other gross lesions were restricted to the heart where a small flat grayish
167 oval-shaped patch (of about 5 mm in diameter) was revealed on the epicardial surface which
168 extended into the myocardium (Fig. 2a). By light microscopy, no lesions were seen in the gills
169 or kidney. The labial polypoid masses were benign papillomas composed of proliferating
170 connective tissue and covered by a multilayered epithelium. Proliferation of the malpighian
171 cells upon a narrow base of connective tissue without an invasion down into the underlying
172 dermis was also seen (Fig. 1b). The grayish spot on the heart was a bening myoma composed
173 of collections of cross-striated muscle fibres and surrounded by connective tissue (Fig. 2b).

174 Additionally, vacuolization in the hepatocytes, and multifocal hemosiderin deposition in the
175 red pulp of the spleen were revealed.

176 **Virus isolation.** Rounding and detachment of the cells appeared on 12, 14 and 17 days post-
177 inoculation in the tissue cultures incubated at 22°C, 19°C and 15°C, respectively. In the
178 negative controls, no CPE was observed. A few days later, the CPE was complete. However,
179 in the second and third passages, no CPE was observed. PCR testing of samples from the
180 successive passages for the detection of the CV or certain dsDNA viruses (adeno-, herpes-
181 and iridovirus) also remained negative.

182

183 **PCR and sequencing.** All PCRs, performed for the detection of different large DNA viruses,
184 gave negative results. However, the circovirus PCR resulted in specific products from every
185 examined organ (including the gills, liver, spleen and kidney) as well as from the labial
186 proliferative tissue. Interestingly, the gills, the spleen, and the labial tissue all initially
187 displayed a heavy longitudinal smear and discrete bands of amplicons were obtained after
188 only 10× dilution of the target DNA solutions. The size of the identical amplicons was found
189 to be 303 bp after editing out the primer sequences. By homology search, the conservative
190 region of the circoviral replication-associated protein (Rep) was identified. The inverse nested
191 PCR yielded an 1192 bp DNA fragment. It was first sequenced directly, then it was cloned
192 and sequenced again for a better quality outcome. This fragment contained the whole *cap*
193 gene, the rest of the *rep* gene with the two intergenic regions.

194 **Genome sequence analysis.** The complete genome of the putative eel CV was found to
195 encompass 1378 nt with an average G+C content of 48.7%. The full genome sequence was
196 deposited to the GenBank and assigned to accession number KC469701. The genome
197 organization was found to be somewhat divergent from one typical of CVs, inasmuch as the
198 putative *cap* gene proved to be significantly shorter (Fig. 3). Nonetheless, two oppositely

199 oriented major ORFs which flanked a putative stem-loop element were found. The stem-loop
200 element, situated in the 5' intergenic region, possessed 12-bp stem. Its loop region consisted
201 of 12 nucleotides with the conserved circoviral nonamer sequence (TAGTATTAC) therein as
202 shown in Fig. 4. The deduced product of the *rep* gene was predicted to consist of 286 amino
203 acid (aa) residues containing the conserved RNA helicase domain. Its closest homologue in
204 the GenBank, with 51% aa identity, was the corresponding region of the CV recently
205 described in the wels catfish (Lőrincz et al. 2012). The other ORF, supposedly corresponding
206 to the gene of the capsid protein (Cap), was predicted to code for 114 aa only. This protein did
207 not show convincing homology to any proteins in the GenBank. Nonetheless, similar to the
208 Cap proteins of other known CVs, a 32 aa-long arginine-rich stretch
209 (RRRVYRRKSNRRPIRNCQRRYRRPIRRERNNR) was found close to the N terminus. The
210 length of the intergenic regions between these ORFs was 107 and 65 nt, at their 5' and 3'
211 termini, respectively. Two additional, oppositely oriented ORFs overlapping the *rep* gene
212 were also found. One of these, in the same orientation as the *rep* gene, encompasses 309 nt,
213 the translated aa sequence shows homology to a small hypothetical (17 kDa) protein of the
214 beak and feather disease virus (Niagro et al. 1998). The other ORF, in a reverse position
215 compared to *rep*, consists of 483 nt. Its deduced product shows homology to hypothetical
216 genes described from pigeon and duck CVs (Mankertz et al. 2000; Chen et al. 2006). It has to
217 be mentioned however, that this 483-nt ORF has no ATG. A possible alternative start codon
218 could be the ATA triplet (in nt position 19 to 21). If so, then the predicted protein product
219 would consist of 155 aa.
220 **Quantitative PCR.** With qPCR, the virus was successfully detected in all five of the
221 examined organs. The statistical mean of five parallel qPCRs for the viral genome resulted in
222 the following cycle threshold (Ct) values: liver 23.6, spleen 23.9, gills 26.0, kidney 27.5,
223 stomatopapilloma 28.1. It means that the relative amount of the viral DNA in the examined

224 organs is the following: 1 unit in the tissues of the stomatopapilloma, 1.25 unit in the kidney,
225 20 units in the gills, 130 units in the spleen, and 157 units in the liver.
226
227 **Phylogenetic analysis.** The phylogeny reconstruction made by the distance matrix analysis
228 on complete deduced Rep sequences from 27 CVs is presented in Fig. 5. The alignments
229 consisted of 229 aa. The newly detected putative European eel CV appeared in the clade of
230 the *Circovirus* genus within the *Circoviridae* family as a sister group of the wels catfish CV
231 (Lőrincz et al. 2012). However, the monophyly of fish CVs was not confirmed.
232
233 **Discussion**
234 In the present work, the full genome sequence of a putative novel CV was determined.
235 The virus originated from a European eel showing typical signs of the so-called cauliflower
236 disease. We propose to name this virus the European eel circovirus (EeCV). The isolation of
237 the virus on an eel kidney cell line failed. The CPE, observed after the first inoculation, did
238 not appear again in the consecutive passages. We speculate that the cell degeneration was
239 probably due to a direct cytotoxic effect by some material or unknown, uncharacterized virus
240 that was not detectable by our PCRs.
241 Apart from the heart myoma and the stomatopapillomas, which were probably large
242 enough to interfere with feeding, no other significant pathological alterations were revealed. It
243 seems that the examined eel was caught in a viraemic status, since every examined organ
244 contained circoviral DNA. The initial PCR with undiluted samples of the stomatopapilloma,
245 gills, and spleen resulted in a heavy smear. This signaled the possibility of too high
246 concentration of the total DNA. Indeed, the 10-fold dilution of the same samples produced
247 discrete bands of the expected size.

248 Thanks to the significant improvement in the efficiency of detection methods (Halami et
249 al. 2008), an incredible increase in the number of the known small circular ssDNA viruses has
250 occurred in the second half of the past decade. The known host range of CVs (including
251 mainly birds and swine) has also grown rapidly (Delwart & Li, 2012). Besides a couple of
252 additional mammals, a number of invertebrate hosts have also been found to harbor seemingly
253 specific CVs. The first CVs from lower vertebrates have been published most recently. These
254 viruses, detected in barbel (*Barbus barbus*) and wels catfish (*Silurus glanis*), have been
255 characterized by full genome analyses (Lőrincz et al. 2011 & 2012). Additional putative
256 piscine CVs have been discovered by PCR in other fishes, as well as in two species of
257 amphibians. But, these CVs have not been confirmed yet by complete genomic sequences
258 (Fehér et al. 2013; Tarján et al. 2014). Although the barbel and wels catfish CVs have been
259 found during investigations for the cause of increased mortality, the role of these viruses in
260 disease could not be confirmed.

261 In this case, a direct connection between the presence of the putative circoviral DNA
262 and the cauliflower disease could be revealed. The porcine and most avian CVs are known to
263 have an immunosuppressive effect (Todd 2004), which may exacerbate the pathogenicity of
264 certain concurrent infectious agents. Nonetheless, it is also possible that CVs can cause
265 generalized infection in individuals whose immune system is temporarily or permanently
266 impaired. Ng et al. (2009) have drawn similar conclusions when they examined a novel small
267 ssDNA virus in sea turtles with fibropapillomas.

268 The taxonomy of EeCV is somewhat ambiguous. According to the phylogeny
269 reconstruction based on the Rep sequence, the piscine (barbel and wels catfish) CVs seem to
270 form a common clade with an interesting small circular ssDNA virus. This common clade is
271 formed with NG13, which was recently discovered by screening human fecal samples
272 collected in Nigeria (Li et al. 2010). The NG13 virus had originally been described as an

273 outlier to the proposed new genus *Cyclovirus* (Li et al. 2011) however, its exact taxonomic
274 place is still unclear. The nonamer sequence of the stem-loop of the NG13 is identical to that
275 of the CVs, but its genome organization resembles that of the cycloviruses (CyVs) (Delwart
276 & Li, 2012). Our analysis shows that NG13 and fish CVs might share a close evolutionary
277 origin. It seems that the EeCV does not form a monophyletic group with the other two fish
278 CVs. A possible reason for such a result could be the incomplete taxon representation.
279 Perhaps the full genome analyses of additional novel CVs from fishes and other lower
280 vertebrates will help discern the phylogenetic relationships more precisely in the future.

281 The family *Circoviridae* is currently facing a radical revision. It has been proposed that
282 the *Gyrovirus* genus be moved to the *Anelloviridae* family. Furthermore, the establishment of
283 a new genus, *Cyclovirus* within the *Circoviridae* has also been proposed (Biagini et al. 2013).
284 There are however, numerous circovirus-like viruses with unknown ancestry at the moment.

285 Based on its genome organization, the putative EeCV most closely resembled the
286 members of the *Circovirus* genus. It encodes a Rep protein on the virus sense strand and a
287 putative Cap protein in the opposite direction. The presence of the two additional smaller
288 ORFs exhibit a clear homology to the hypothetical genes of yet unknown function, described
289 in pigeon (Mankertz et al. 2000) and duck CVs (Chen et al. 2006), and in the beak and feather
290 disease virus (Niagro et al. 1998). These homologies further confirm the place of EeCV in the
291 same genus. Furthermore, the stem-loop structure, which is involved in the initiation of the
292 viral genome replication (Steinfeldt et al. 2001), had a typical circoviral nonamer sequence in
293 EeCV, identical to that of the fish CVs, porcine CV1, pigeon, gull and finch CVs (Li et al.
294 2010). The lengths of the intergenic regions between the *rep* and *cap* genes also show
295 differences between CVs and CyVs (Li et al. 2010). These regions in EeCV are also
296 characteristic for CVs. Its closest evolutionary relative is the wels catfish CV.

297 Interestingly, the exceptionally short *cap* gene of EeCV did not show obvious homology
298 to its counterparts in any known CVs or CyVs. Yet, a characteristic feature of the circoviral
299 Cap proteins, namely the arginine-rich stretch close to the N-terminus, was identified.
300 It is intriguing to speculate about a recombination event between a progenitor and an
301 unknown circular virus resulting in EeCV. Among ssDNA viruses (even originating from
302 distantly related virus families), recombination events occur frequently (Gibbs & Weiller,
303 1999; Martin et al. 2011). Moreover, a recombination between a circo-like virus and ssRNA
304 virus has also been hypothesized (Diemer & Stedman, 2012). At the moment however, the
305 origin of the cap gene of EeCV remains enigmatic since the BLAST searches did not find any
306 reliable homology to any known sequences among the environmental samples.
307 According to the proposed new rules of the Circoviridae Study Group of the ICTV, the
308 comparative analysis of the complete genomes would replace the earlier practice of
309 comparing the *cap* sequences in the establishment of new species (Biagini et al. 2013).
310 Considering the demarcation criteria, EeCV unequivocally represents a novel species.
311 At the discussion of the qPCR results, we emphasize that these results are based on the
312 examination of one specimen and at one time-point. Thus, these data should be considered as
313 preliminary results. The qPCR confirmed the results of the conventional PCR, inasmuch as all
314 examined organs contained the viral DNA suggesting that the examined eel was caught in a
315 viraemic status. The relative amount of the viral DNA may indicate that the viral replication
316 takes place in the liver and spleen.
317 To examine the pathogenicity of EeCV in experimental infections, the isolation of the
318 virus would be essential. Nonetheless, screening eels, especially those showing clinical signs
319 of the cauliflower disease, for the presence of cicoviral DNA should also be continued.
320

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328

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428 FIGURE LEGENDS
429
430 **Figure 1a.** *Anguilla anguilla***.** Clinical appearance and microstructure of the cauliflower
431 disease. (A) Bilateral labial polipoid masses in the eel from the Lake Balaton. (B)
432 Microphotograph of the labial mass composed of proliferating connective tissue covered by
433 multilayer epithelium. Proliferation of the malpighian cells upon a narrow base of connective
434 tissue without invasion down into the underlying dermis.

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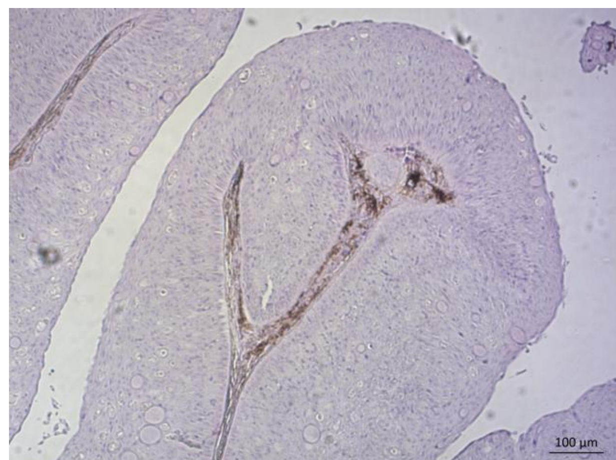

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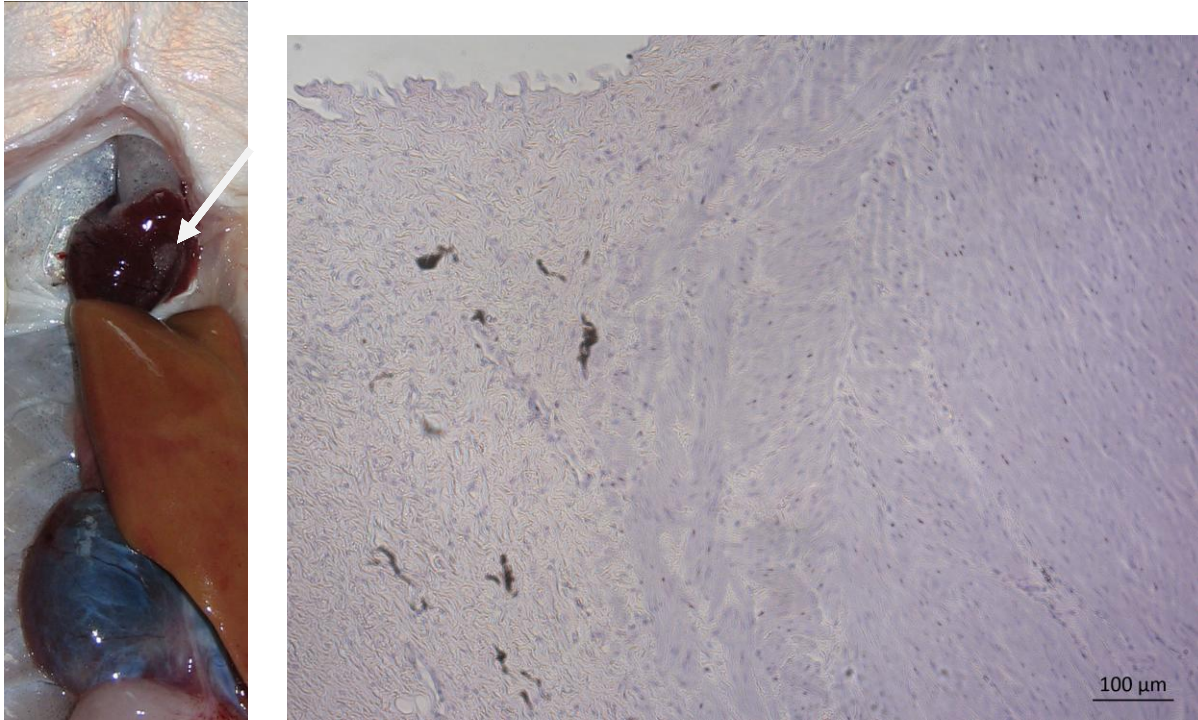
437 **Figure 2a.** Macroscopic picture of the abdominal cavity. The white arrow points to the lesion

438 on the epicardium. (B) Microphotograph of the benign myoma composed of collections of

439 cross-striated muscle fibres and surrounded by connective tissue.



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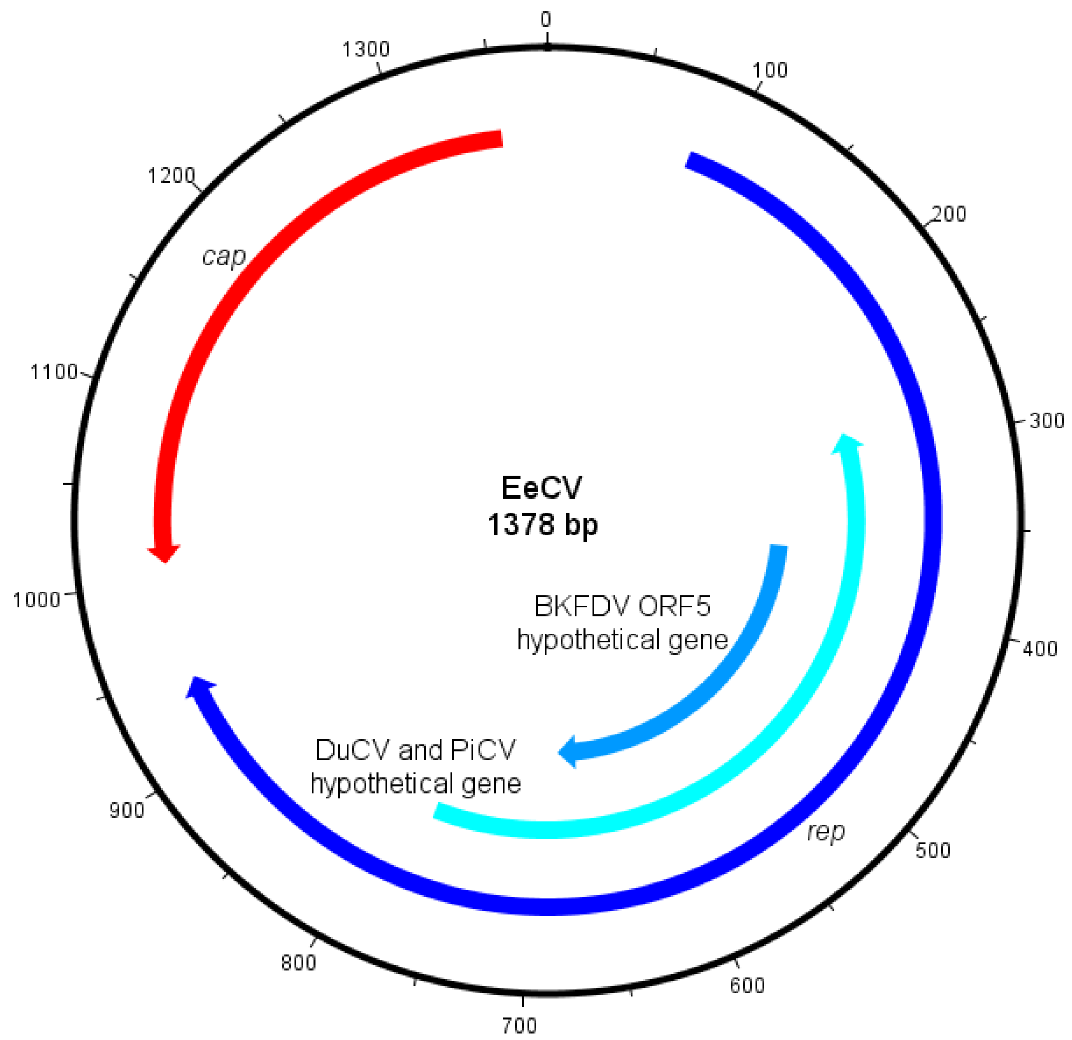
442 **Figure 4.** Genome organization of the EeCV. Besides the two major ORFs, there are two

443 additional hypothetical ORFs. One of them (BKFDV ORF5) shows similarity to the ORF 5 of

444 Beak and feather disease virus (BKFDV). The other (DuCV and PiCV) is homologous to

445 hypothetical ORFs of duck (DuCV) and pigeon (PiCV) CVs.



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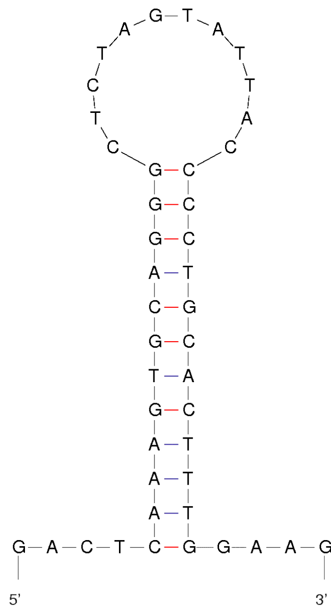
447 **Figure 4.** The structure and sequence of the putative stem-loop



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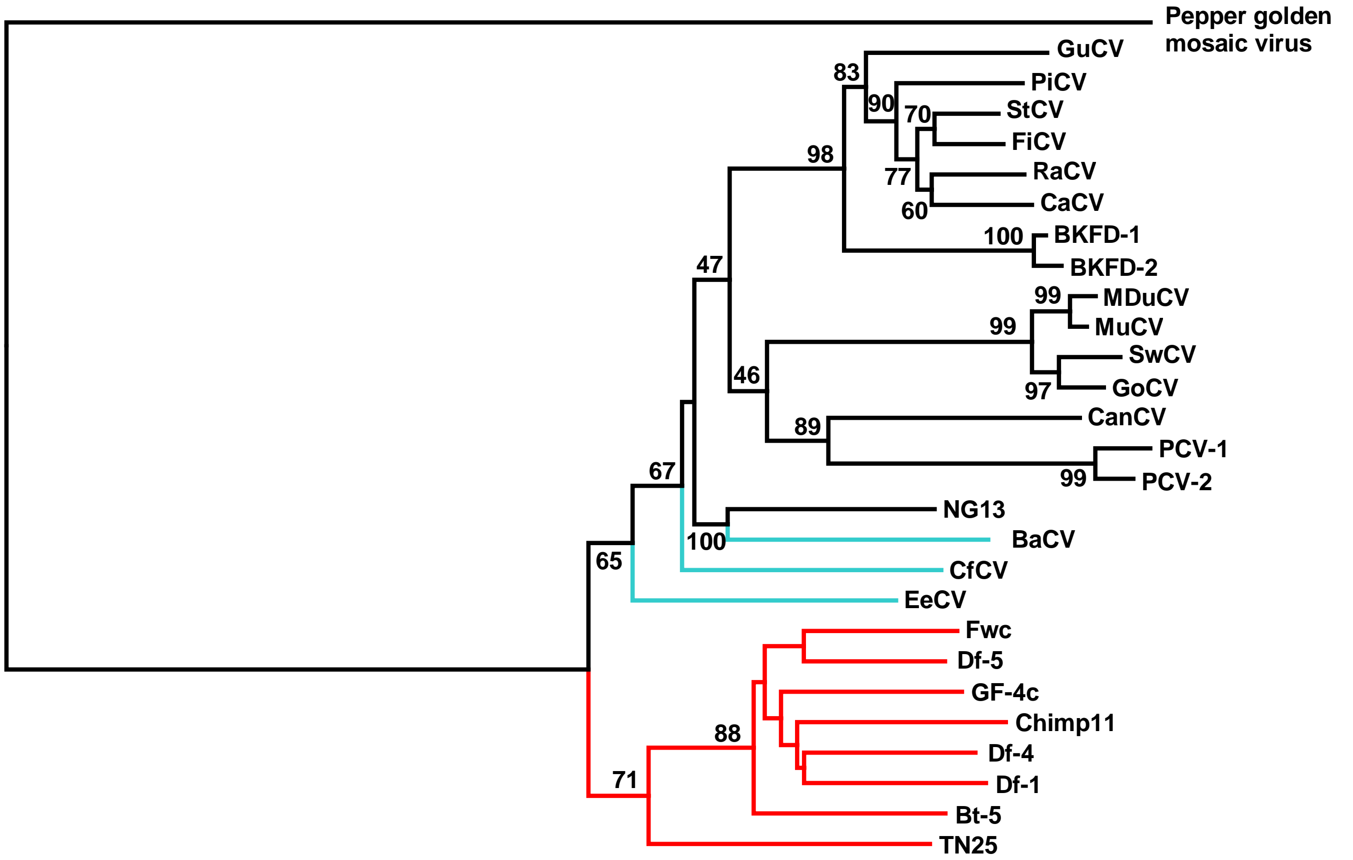
450 **Figure. 5.** Phylogenetic tree reconstruction based on the distance matrix (JTT model) of the
451 deduced amino acid sequences (229 aa) of the rep genes of ssDNA viruses. Bootstrap values
452 are shown on the branches. The cycloviruses, the circoviruses and the fish circoviruses within
453 the family *Circoviridae* are designated by different colored lines on the tree. Abbreviations:
454 GuCV=gull circovirus; PiCV=pigeon circovirus; StCV=starling circovirus; FiCV=finch
455 circovirus; RaCV=raven circovirus; CaCV=canary circovirus; BKFD-1=beak and feather
456 disease virus 1; BKFD-2=beak and feather disease virus 2; MDuCV=muscovy duck
457 circovirus; MuCV=mallard circovirus; SwCV=swan circovirus; GoCV=goose circovirus;
458 CanCV=canine circovirus; PCV-1=porcine circovirus 1; PCV-2= porcine circovirus 2;
459 NG13=human stool associated circovirus; BaCV=barbel circovirus; CfCV=catfish circovirus;
460 EeCV=European eel circovirus; Fwc= Florida woods cockroach-associated cyclovirus; Df-
461 5=dragonfly cyclovirus 5; GF-4c=bat cyclovirus; Chimp11=chimpanzee Cyclovirus 11; Df-
462 4=dragonfly Cyclovirus 4 ; Df-1=dragonfly cyclovirus 1; Bt-5=bat feces cyclovirus ;
463 TN25=human feces cyclovirus



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