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Molecular characterisation of novel circoviruses from finch and gull

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Molecular characterisation of novel circoviruses from finch and gull

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Abstract:

The purpose of this study was to molecularly characterise circoviruses that infect finch and gull. Circovirus-specific DNAs were isolated using PCR methods from bursa of Fabricius tissues from a Gouldian finch (*Chloebia gouldiae*) and a herring gull (*Larus argentatus*) that were known to be circovirus-infected. Nucleotide sequence determination and analysis of cloned genomic DNAs showed that these circoviruses represented novel members of the genus *Circovirus* of the family *Circoviridae*, and have been tentatively named Finch circovirus (FiCV) and Gull Circovirus (GuCV). Both new circoviruses shared genome organisational features with previously characterised circoviruses, such that both contained 2 major, inversely-arranged open reading frames (ORFs) encoding the putative replication associated and capsid proteins, and both contained a potential stem-loop and nonanucleotide motif. Phylogenetic analyses based on genome nucleotide sequences and involving the 7 additional genus members indicated that FiCV and GuCV were more closely related to canary circovirus (CaCV), beak and feather disease virus (BFDV) and pigeon circovirus (PiCV), and that FiCV and CaCV were the most closely related avian circoviruses. Pairwise comparisons showed that the capsid proteins of FiCV and GuCV shared highest amino acid identity values with those of CaCV (62.0%) and PiCV (40.6%) respectively. The 5' intergenic region of GuCV was longer (207 nucleotides) and contained more direct and inverse repeated

sequences than those of other circoviruses, while the 3' intergenic region of FiCV was notable in being longer (307 nucleotides) than its counterparts in other circoviruses and in containing 2 long repeats of 77 nucleotides.

Introduction

Circoviruses are small non-enveloped icosahedral viruses (diameter 15-20 nm) with circular, covalently-closed, single-stranded DNA genomes (1.7-2.3 kb). The family Circoviridae contains 2 genera. Chicken anaemia virus (CAV) is the only member of the genus Gyrovirus, while the genus Circovirus currently comprises porcine circoviruses types 1 and 2 (PCV1, PCV2), psittacine beak and feather disease virus (BFDV), pigeon circovirus (PiCV), canary circovirus (CaCV), goose circovirus (GoCV) and duck circovirus (DuCV) (Todd et al. 2005). Members of the Circovirus genus differ from CAV with regard to both particle size and morphology (Crowther et al. 2003) and with regard to genome organisation and expression strategy. Thus, CAV has a negative sense genome organisation and uses 1 unspliced polycistronic transcript, whereas the other circoviruses have distinctive ambisense genome organisations, and, based on studies on PCV1, several spliced transcripts are likely to be used (Todd et al. 2005).

Avian circovirus infections are diagnosed by histology, electron microscopy, in situ hybridisation (ISH) and, increasingly by PCR. One of the characteristic histological features of circovirus infections of young birds is the occurrence of cytoplasmic globular or botryoid inclusions, which are commonly found in mononuclear inflammatory cells in the bursa of Fabricius. The most studied avian viruses of the circovirus genus are BFDV, which causes feather and beak

abnormalities in a wide range psittacine birds, and PiCV, infections with which are associated with a variety of clinical signs including lethargy, weight loss, respiratory distress, diarrhoea and poor race performance (Woods et al. 1993; Shivaprasad et al., 1994). Infections with BFDV and PiCV are both associated with lymphoid depletion and immunosuppression, which appears to increase the severity of secondary infections. Circoviruses have been detected in farmed geese and ducks with growth and feathering problems but the clinico-pathological significance and economic importance of circovirus infections of these birds remains unknown (Hattermann et al., 2003; Soike et al., 1999, 2004.)

Little is known about the roles of circoviruses in causing disease in passerine species such as canaries and finches. Circoviruses have been reported to cause a condition known as black spot in neonatal canaries in Europe (Goldsmith, 1995). Later, a novel circovirus, named canary circovirus (CaCV) was detected in adult canaries that died after a short illness characterised by dullness, anorexia and feather abnormalities (Phenix et al. 2001; Todd et al., 2001). Circovirus infection of finches was first reported in the USA in 3- to 6-month-old zebra finches affected by feather loss (Mysore et al., 1995). More recently, Shivaprasad et al. (2004) described the diagnosis by histology, electron microscopy and ISH of circovirus infection in a 10-week-old Gouldian finch (*Chloebia gouldiae*), which had nasal discharge, dyspnoea, anorexia and depression. With the exception of psittacines, there has only been one report to date of possible circovirus infection in free-living birds. In that report, Twentyman et al. (1999) described the finding of circovirus-like particles in a diseased southern black-backed gull (*Larus dominicanus*) found in New Zealand. In situ hybridisation subsequently confirmed the presence of circovirus in samples from the southern black-backed gull reported by Twentyman et al. (1999) and in herring

(*Larus argentatus*) and black-headed (*Larus ridibundus*) gulls from Sweden (Smyth et al. in press).

The present paper describes the molecular characterisations of novel circoviruses from the Gouldian finch and herring gull using nucleotide sequence determination and comparative sequence analysis.

Materials and Methods

Samples. Bursa of Fabricius (BF) tissue samples were collected from the 10-week-old Gouldian finch (*Chloebia gouldiae*) (Shivaprasad et al., 2004), and a young herring gull (*Larus argentatus*), which had been shown to be infected with circoviruses by ISH using a circovirus-specific probe (Smyth et al., in press).

DNA Extraction. Viral DNA was extracted from approximately 25 mg BF tissue using the QIAmp DNA mini kit (Qiagen, Westburg, Holland), according to the manufacturer's directions.

Polymerase chain reaction (PCR). DNA extracts were subjected to PCR using primers CAN-1 F (5' – GGA GCT GTT GCC GCC GTG A – 3') and CAN-1R (5' – TAC CCA TCC CAC CAG TCA CC – 3'). These primers flank a region of approximately 250 nt in the Rep gene of CaCV and were selected because highly conserved nucleotide (nt) sequences occur in the genomes of 3 PiCV isolates and 7 BFDV isolates. Amplification was performed using the Qiagen HotStart Taq according to the manufacturers' instructions. The cycling conditions comprised an

initial denaturation at 94°C for 15 min followed by 30 cycles of 94°C for 30 sec, 57°C for 45 sec and 72°C for 1 min and then a final extension at 72°C for 7 min.

The complete circular FiCV and GuCV genomes were separately amplified by PCR as linear fragments by PCR using pairs of inverse primers, which were selected following examination of the sequences of the fragments amplified using the CAN-1F and CAN-1R primer sets. The inverse primers used to amplify the FiCV genome were Finch AF (5' - GCT TTC ACG GCG GCA ACA GC - 3') and Finch AR (5' - CGG AAG AGG TAT GGC CGA AGT C - 3') and those used to amplify the GuCV genome were Gull AF (5' - TTC ACT CAT TTG ACT TCC GC - 3') and Gull AR (5' - ATC GCG CGA GAG TTC AGT GA - 3'). The amplification conditions for the FiCV and GuCV genomes were an initial denaturation at 94°C for 15 min followed by 30 cycles of 94°C for 30 sec, 60°C for 30 sec (FiCV) or 43°C for 30 sec (GuCV) and 72°C for 2min 30 sec and a final extension of 7 min.

Cloning of circovirus-specific DNA fragments. FiCV- specific and GuCV- specific PCR products of approximately 250nt that were amplified by the CAN 1F and 1R primers from DNA extracts of finch and gull BF tissue samples were cloned using the pCR2.1[®]TOPO vector kit (Invitrogen, UK). The same cloning vector was used to clone PCR products of approximately 2kb that were amplified using the inverse primer sets specific to FiCV and GuCV.

Nucleotide sequence determination and analysis. Cloned circovirus-specific DNAs were sequenced (ABI PRISM dye terminator ready reaction kit), initially using the M13 primers located on the cloning plasmid, and then using a primer walking strategy. Sequencing was performed at least twice in each direction, with 2 cloned

genomic fragments being sequenced for both FiCV and GuCV. Nucleotide (nt) sequences were analysed using MacDNASIS and the Vector NTI software suite and similar searches were performed by the BLAST program using the non-redundant nucleic acid sequence database at the NCBI, USA. Sequence alignments were performed using the Clustal W (University of Wisconsin Genetics Group version 8) programmes (Thompson et al., 1997). Data sets were statistically analysed and phylogenetic trees were constructed using the PHYLIP software package (Department of Genetics, University of Washington, USA). The EMBL Nucleotide Sequence Database Accession Numbers of the PiCV, GoCV, CaCV and DuCV sequences are AJ298229, AJ304456, AF346618 and AY228555. Additional sequences used for comparison purposes were BFDV (AF080560), PCV1 (U49186) and PCV2 (AF055392).

Accession numbers. The Genbank accession numbers of the GuCV and FiCV genomes are DQ845074 and DQ845075 respectively.

Results

Isolation, cloning and nucleotide sequence determination of FiCV and GuCV genomes.

PCR, performed using the CAN-1F and CAN-1R primers, resulted in the amplification of products of approximately 250 nt from DNA extracts of known circovirus-infected finch and gull BF tissues. Based on the CaCV genome sequence, these products were of the size expected for circovirus DNAs. Nucleotide sequence determinations, performed with cloned fragments, showed that the amplicons shared high levels of nt identity with the CaCV genome and allowed the design of specific pairs of inverse primers that were capable of amplifying complete circovirus genome as linear fragments of approximately 2 kbp (data not shown).

Sequencing of the cloned genome fragments generated by inverse primer -PCR showed that the FiCV and GuCV genomes were 1962 and 2035 nt respectively. The two FiCV clones sequenced differed at 7 nt positions and these resulted in 2 amino acid changes in both the Rep and Capsid proteins. The two GuCV clones sequenced were identical. The FiCV genome shared nt identity values of between 48.8% (PCV2) and 65.1% (CaCV) with other members of the genus *Circovirus*, while the GuCV genome shared nt identity values of between 48.6% (PCV1) and 59.6% (PiCV).

Genome organisation of FiCV and GuCV. Nucleotide sequence analyses showed that FiCV and GuCV had genome organisations that were similar to those of previously characterised circoviruses such as PCV2, the type species of the genus, and PiCV (Figure 1). This included the possession of a distinctive inverted repeat sequence with the potential to form a stem-loop that contains at its apex the highly conserved nonanucleotide motif at which rolling circle replication (RCR) of the virus DNA strand is postulated to initiate. In the case of FiCV and GuCV this nonanucleotide sequence was TAGTATTAC. By convention, the nt numbering scheme for circovirus genomes places the A residue, which occurs in the eighth position in the nonamer motif, as the first nucleotide of the genome sequence.

Two major reading frames (ORFs), which have been labelled V1 and C1 in accordance with the convention used previously with circoviruses, have similar locations in the FiCV and GuCV genomes and in the genomes of other members of this genus. The V1 ORF, located on the virus sense strand, is postulated to encode the replication associated protein (Rep), while the C1 ORF, which is located on the complementary sense strand, is postulated to encode the capsid protein. The initiation codons of both FiCV ORFs and the V1 ORF of GuCV were identified as being ATG. No suitably located ATG was identified at the start of the GuCV C1 ORF.

Examination of the sequence showed the presence of other codons, including an ATA (nt 1873-1871), a GTG (nt 1927-1925) and CTG (nt 1882-1880), which are alternatives to ATG. Of these GTG was considered to be the most likely candidate in that it produced higher aa identity values following pairwise comparison of the GuCV C1 ORF with those of other avian circoviruses. Both the FiCV and GuCV genomes contain one other ORF with a coding capacity greater than 100 amino acids. In each case, this ORF, labelled C2, occurs on the complementary sense strand and overlaps the 3' region of the V1 ORF (Figure 1).

Comparative sequence analysis of ORFs. The putative Rep genes of FiCV (nt 29-904) and GuCV (nt 100-1017) encode proteins of 291 amino acids (aa) and 305 aa respectively. These values are within the range 289 aa (BFDV) to 317 aa (PiCV) observed with other circoviruses. The predicted Rep protein of FiCV shares aa identities of between 43.0% (PCV2) and 75.6% (CaCV) with other circoviruses, while that of GuCV shares aa identity values of between 38.2% (PCV2) and 62.4% (CaCV) (Table 1). The Rep proteins of FiCV and GuCV both contain three highly conserved aa motifs which are associated with RCR activity (Ilyina and Koonin, 1992). These include the FTLNN motif, which occurs at aa residues 15-20 in FiCV and at residues 28-33 in GuCV and the T--LQG motif, which occurs at aa residues 50-55 in FiCV and residues 65-70 in GuCV. The sequence of the third RCR motif, CSK, is replaced by CGK in the FiCV (aa residues 91-93) and GuCV (residues 105-109) Reps respectively. A fourth motif, G---GKS, which is putatively associated with dNTPase activity (Desbiez et al., 1995), occurs in both the FiCV (residues 167-173) and GuCV (residues 182-188) Rep proteins. Additional regions of conserved aa sequence are observed when the Rep proteins of FiCV and GuCV are compared with those of other

circoviruses. These include WWDGY, DDFYGW and DRYP sequences, which occur between aa residues 191 and 223 in the FiCV Rep and between residues 206 and 238 in the GuCV Rep.

The putative capsid proteins encoded by C1 ORFs of FiCV and GuCV are 235 and 245 aa respectively, and fall within the range 233 aa (PCV1, PCV2) to 250 aa (PiCV) determined for those of other members of the genus. The predicted FiCV capsid protein shares aa identities of between 21.2% (DuCV) and 62.5% (CaCV) with other circoviruses while the predicted GuCV capsid protein shares values of between 17.1% (GoCV) and 40.6% (PiCV) (Table 1).

Low levels (<23%) of aa identity were found when the C2 ORFs of FiCV and GuCV were compared in a pairwise manner with those of other avian circoviruses (data not shown). However, a Blast search revealed that a 77 amino acid region largely corresponding to the C terminal half of the FiCV C2 ORF exhibited 43% aa identity with the C2 ORF of PiCV.

Intergenic regions. In circovirus genomes, the 5' intergenic region is regarded as the region occurring between the initiation codons of the major C1 and V1 ORFs, with the 3' intergenic region occurring between the termination codons of these ORFs. The 5' intergenic region of FiCV is 71 nt in length and is smaller than those of other viruses from this genus (Table 2), while that of GuCV is 207 nt and is larger than those of other circoviruses. The conserved nonanucleotide motif of GuCV is flanked by a potential stem-loop comprising ten G-C base pairs, while that of FiCV is flanked by a potential stem loop comprising 15 base pairs. Two direct tandem repeat sequences comprising 8 or 9 nucleotides, which occur as part of or adjacent to the right-hand side of the potential stem loop, are found in the avian members of this

genus. On the basis of studies performed with the PCVs, such direct tandem repeats are considered to be sites at which the Rep proteins bind the replicative form (RF) DNA prior to initiating RCR. In FiCV each of these repeats comprise 7 nt (TGGAACC), while the repeat sequences of the GuCV comprise the octonucleotide GGGGCCAT. The large 5' intergenic region of GuCV contains additional direct and inverted short repeats of 8 nucleotides, while that of FiCV possesses only one additional inverted repeat (Figure 2).

The 3' intergenic region of FiCV is 307 nt and is considerably longer than those of other circoviruses (range 36 to 249 nt) while that of GuCV is 72 nt. The 3' intergenic region of FiCV contains 2 long repeats of 77 nt.

Phylogenetic analysis. Examination of the phylogenetic tree based on the complete genome nucleotide sequences showed that the mammalian circoviruses, PCV1 and PCV2, were more distantly related to avian members of the genus (Fig. 3). GoCV and DuCV were shown to be closely related to each other and clearly separate from the remaining 5 avian circoviruses. The phylogenetic analysis further showed that BFDV and PiCV were closely related to each other and distinct from the cluster containing GuCV, CaCV and FiCV. Of these, FiCV was shown to be most closely related to CaCV.

Discussion

Although earlier in situ hybridization investigations indicated that finches and gulls were infected with circoviruses (Shivaprasad et al. 2004; Smyth et al. in press), there was uncertainty as to whether these birds were infected with novel circoviruses or

with previously characterised circoviruses. The nt sequence-based molecular characterisations reported in this study confirms that the circoviruses infecting the finch (*Chloebia gouldiae*) and herring gull (*Larus argentatus*) were novel and should be recognised as new members of the genus *Circovirus*. Using the convention adopted previously with other viruses of the genus, they have been tentatively named Finch circovirus (FiCV) and Gull circovirus (GuCV). This paper also provides additional knowledge about the likely host range of avian circoviruses and additional nt sequence data on which the development PCR diagnostic tests can be based.

To date, there have been no reports describing the cell culture growth of avian viruses of the circovirus genus and no attempts have been made to grow FiCV and GuCV in cell culture. The absence of in vitro propagation methods has prevented these viruses from being characterised by more traditional serological methods such as cross-neutralisation and cross-immunofluorescence assays. As a consequence virus identification and characterisation has depended on molecular approaches including PCR, nt sequence determination and comparative sequence analysis. Sequence analyses performed in this study confirmed that the FiCV and GuCV genomes displayed features that are considered to be characteristic of viruses of the genus *Circovirus* by the International Committee for the Taxonomy of Viruses (ICTV) (Todd et al. 2005). These features include the possession of the distinctive potential stem-loop and conserved nonanucleotide motif at which RCR is postulated to initiate and major V1 and C1 ORFs. The FiCV and GuCV genomes also met the species demarcation criteria suggested by ICTV, in that they shared genome nt identities of less than 75% and capsid protein aa identities of less than 70% with previously characterised genus members. As such FiCV and GuCV are likely to be recognised as additional members of the genus.

Although the genome organisations of FiCV and GuCV were very similar to those of other genus members, examination of their 5' and 3' intergenic regions has increased our knowledge about the genetic diversity that these viruses can display. Thus, the 5' intergenic region of GuCV was longer and contained more direct and inverted repeats than those of other circoviruses, whereas its FiCV counterpart was shorter than those of other circoviruses and lacked repeats other than the tandemly-repeated sequences, which occur on the right hand side of the potential stem-loop. Investigations have indicated that the 5' intergenic region of PCV1 largely corresponds to the origin of DNA replication (Mankertz et al. 1997). In the case of PCV1 and PCV2, the Rep and Rep' proteins are postulated to bind to the tandemly-repeated sequences adjacent to the right side of the potential stem loop prior to cleavage of the nonanucleotide motif present in the virus strand (Mankertz et al. 2003). Comparatively little is known about the 3' intergenic regions of circoviruses, including the PCVs. In both of these mammalian circoviruses, the 3' intergenic regions are very short (36 and 38 nt respectively) in comparison to those found in some of the avian examples including CaCV (249 nt), BFDV (234 nt) and DuCV (232 nt). The newly characterised FiCV has the longest 3' intergenic region observed so far and differs from other circoviruses in having 2 long repeats of 77 nt. Repeated sequences are not common feature of these regions, although DuCV is exceptional in having 4 repeats each of 44 nt. The functions of these long repeats and the shorter repeats, both direct and indirect, which are found in the 5' intergenic regions of viruses such as GuCV, BFDV and CaCV remain to be determined. However, in the absence of cell culture propagation systems, elucidating the roles of these repeats is not likely to be possible. Another unresolved question relating to circovirus molecular biology concerns whether any of the minor ORFs possessed by avian

circoviruses encode functional proteins. Recent research with PCV2 has identified a minor ORF, positioned on the complementary sense strand, that appears to cause the apoptosis, which is observed in PCV2-infected pig kidney cell cultures (Liu et al., 2005). The minor C2 ORFs specified by FiCV and GuCV do not share significant aa identity with this PCV2 ORF. However, an aa identity of 43% was found when a 77 aa region corresponding to the C-terminal half of the C2 ORFs of FiCV was compared with an N-terminal region of the PiCV C2 ORF. The possibility that the minor ORFs specified by particular avian circoviruses encode functional proteins cannot be discounted.

A phylogenetic tree based on genome nucleotide sequences indicated that FiCV and GuCV were part of a grouping of 5 avian circoviruses, which also comprised BFDV, PiCV and CaCV. Within this grouping, GuCV and FiCV were more closely related to CaCV than to BFDV and PiCV, with FiCV and CaCV being the most closely related avian circoviruses. These findings are consistent with the results of the pairwise aa identity comparisons involving both the Rep and capsid proteins (Table 1). The close relationship between FiCV and CaCV is not unexpected because their hosts, canary and finch, are both passerine species. Since the submission of this paper, there have been reports describing the characterisation of two additional novel avian circoviruses, starling circovirus (StCV) (Johne et al. 2006) and raven circovirus (RaCV) (Stewart et al. 2006), both of which appear to be more closely related to CaCV than other avian circoviruses. Although isolates of BFDV are known to infect over 40 types of psittacine birds, findings obtained with the remaining avian circoviruses support the view that these viruses have relatively narrow host ranges and that they may have co-evolved with their avian hosts. Whether other

types of finch and gull are infected with taxonomically different circoviruses remains to be determined.

To date, the majority of animal species, known to be infected with viruses of the circovirus genus, are avian. The comparatively large number (6) of circoviruses characterised since 2000 reflects the likelihood that, due to their narrow host range, (i) many different avian circoviruses exist, (ii) circovirus infections are prevalent within particular avian species, (iii) there is greater awareness of their existence among those investigating avian diseases and (iv) effective PCR-based methods are available for genome isolation. In the present paper, the isolations of the FiCV and GuCV genomes were based on a two-step PCR methodology. The first PCR step capitalised on the probability that nt sequences present in the CaCV genome, which were highly conserved when isolates of PiCV, BFDV and CaCV were compared, would also occur in the genomes of circoviruses that infect finches and gulls and that, as such, PCR amplification of circovirus amplicons would be facilitated. Earlier investigations used degenerate primers, selected after comparison of the PCV1 and BFDV genome sequences, to amplify small regions of the Rep genes of PiCV, GoCV and CaCV (Todd et al., 2001; Phenix et al., 2001; Hattermann et al., 2003). This is the first reported use of the CAN-1F/ CAN-IR primer pair, and the success achieved suggests that these primers may be useful for detecting other avian circoviruses that are more closely related to BFDV, PiCV and CaCV. The second PCR step, involving the use of inverse primers to amplify complete circular genomes as linear fragments, has been previously reported (Todd et al., 2001). The accumulation of sequence data from an increasing range of avian circoviruses will not only be of value in the diagnosis by PCR of particular circovirus species or groups of closely-related species, but, using a degenerate primer PCR approach, may also be valuable in detecting as yet

undiscovered novel circoviruses that may be infecting farmed species such as chickens and turkeys.

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Legends

Figure 1: *Genome organisations of porcine circovirus type 2 (PCV2), pigeon circovirus (PiCV), finch circovirus (FiCV) and gull circovirus (GuCV), showing the locations major open reading frames (ORFs) relative to the potential stem-loop and nonanucleotide motif. The V1 ORF on the virus sense strand encodes the putative replication associated protein (Rep), while the C1 ORF on the complementary sense strand encodes the putative capsid protein. The first and last nucleotides of the ORFs are shown in brackets.*

Figure 2. *5` intergenic regions of the FiCV and GuCV genomes showing the potential stem-loop and nonanucleotide motifs (bold), at which rolling circle replication of the virus DNA strand is postulated to initiate, and a number of direct (underlined) and inverted (arrows) repeated sequences.*

Figure 3: *Phylogenetic tree constructed by the neighbour-joining method based on complete genome nucleotide sequences of circoviruses of the genus Circovirus using PCV1 as an outgroup. Bootstrap values, calculated on the basis of 1000 resamplings, are indicated at the major nodes.*

Table 1. *Pairwise comparison of circoviruses based on amino acid identities shared by V1 (Rep) and C1 (capsid) ORFs*

	% amino acid identity V1 ORF (Rep)									
		PCV1	PCV2	BFDV	PiCV	CaCV	FiCV	GuCV	GoCV	DuCV
PCV1	-	86.0	43.6	41.5	43.3	43.3	39.3	43.8	44.1	
PCV2	65.5	-	43.6	42.3	40.1	43.0	38.2	42.9	44.1	
BFDV	26.0	26.7	-	58.2	60.3	63.2	54.1	46.4	47.9	
PiCV	25.0	26.5	39.9	-	63.1	66.9	58.0	44.7	48.3	
CaCV	28.5	29.4	43.4	40.1	-	75.6	62.4	45.4	47.6	
FiCV	28.0	29.5	42.3	40.7	62.5	-	62.1	49.3	52.2	
GuCV	25.8	26.6	39.2	40.6	35.7	37.7	-	44.3	46.1	
GoCV	29.1	28.2	26.0	22.4	23.6	24.8	17.1	-	82.6	
DuCV	27.1	25.4	24.3	19.0	22.8	21.2	17.7	46.2	-	

Table 2: Summary of data relating to genome features

	Virius Genome	(nt)	5'	intergen. 3'	intergen.	Nona- nucleotid	^a Tandem repeat	Cap	protein Rep	protein
PCV 1	1759	82		36		TAGTATT	CGGCAGC	233	312	
						AC				
PCV 2	1768	83		38		AAGTATT	CGGCAGC	233	314	
						AC	ACCTC			
BFDV	1993	145		234		TAGTATT	GGGGCAC	247	289	
						AC	CG			
PiCV	2037	90		171		TAGTATT	GGACCCA	273	317	
						AC	C			
CaCV	1952	77		249		CAGTATT	GGAGCCA	250	290	
						AC	C			
FiCV	1962	71		307		TAGTATT	TGGAACC	249	291	
						AC				
GuCV	2035	207		72		TAGTATT	GGGGCCA	245	305	
						AC	T			
GoCV	1821	132		54		TATTATT	GTACTCC	250	293	
						AC	G			
DuCV	1996	111		232		TAGTATT	TACTCCG	257	292	
						AC				

Figure 1. Genome organisations of viruses of the genus *Circovirus*

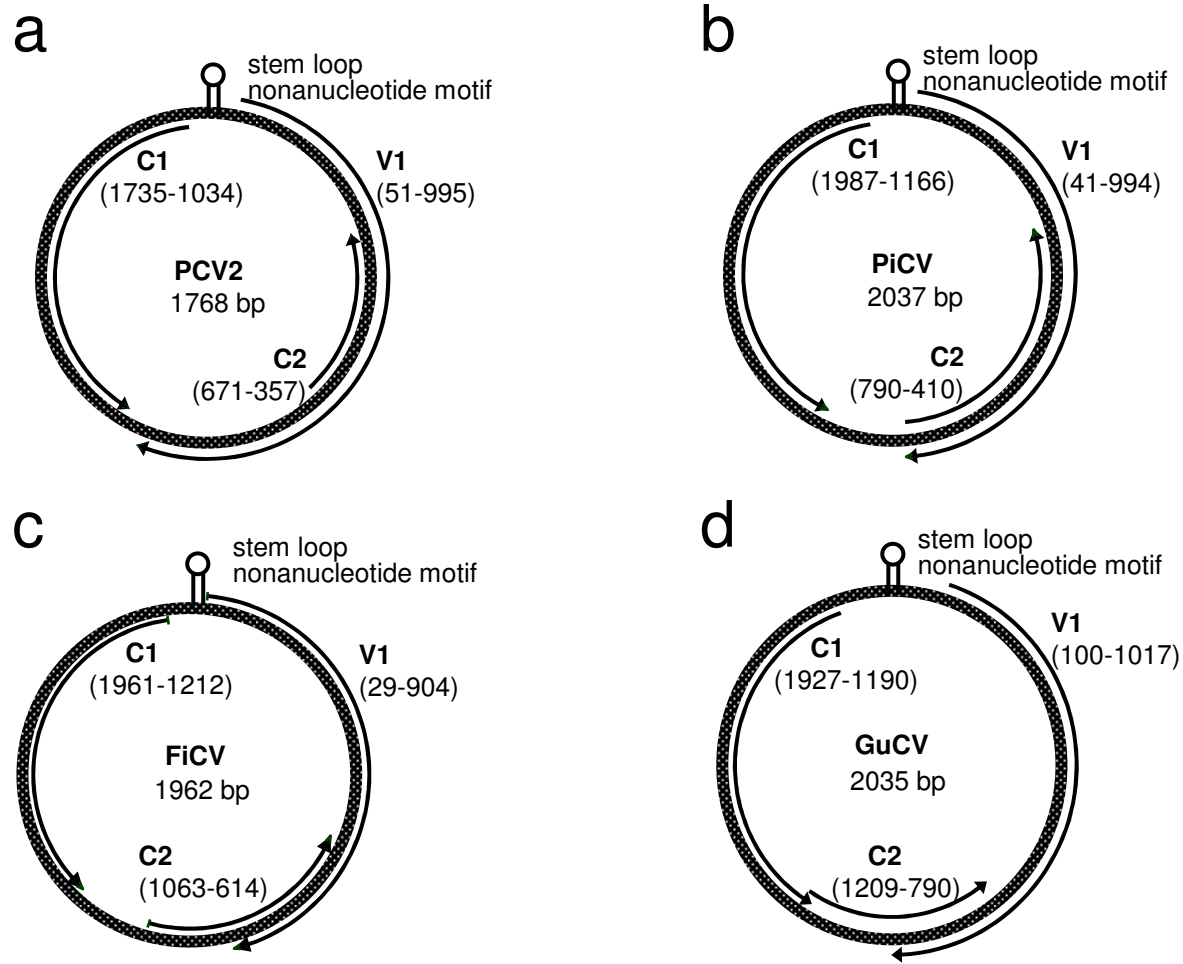


Figure 2. 5' intergenic regions of FiCV and GuCV genomes

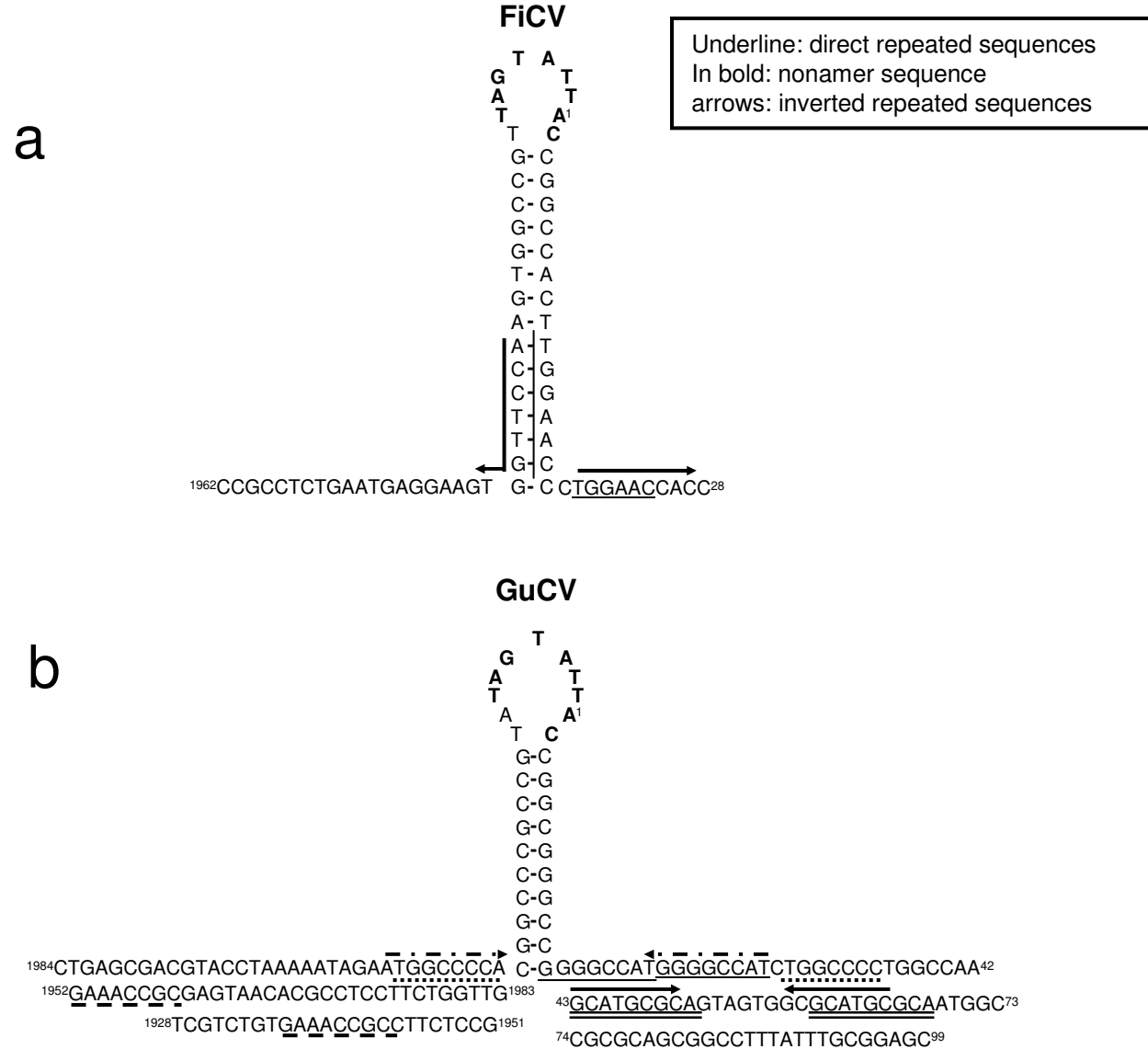


Figure 3. Phylogenetic tree based on genome nucleotide sequences

