Vol. 53: 203–210, 2003 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published February 27

Nucleotide sequence analysis of the glycoprotein gene of putative spring viraemia of carp virus and pike fry rhabdovirus isolates reveals four genogroups

D. M. Stone[1,*], W. Ahne[2], K. L. Denham[1], P. F. Dixon[1], C. T.-Y. Liu[1], A. M. Sheppard[1], G. R. Taylor[1], K. Way[1]

[1]The Centre for the Environment, Fisheries and Aquaculture Science, Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, United Kingdom

[2]Institute of Zoology, Fishery Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Munich, Kaulbachstrasse 37, 80539 Munich, Germany

ABSTRACT: RT-PCR methods have been applied to the detection and sequencing of the glycoprotein gene of putative spring viraemia of carp viruses (SVCV) and pike fry rhabdoviruses (PFRV), including isolates from tench, grass carp, roach, bream and false harlequin, sheatfish and orfe. Phylogenetic analysis of a 550 nucleotide (nt) region of the glycoprotein gene identified 4 groups, I to IV. Significantly, the majority of viruses previously identified as PFRV formed a distinct cluster (Genogroup IV) which shared <80% nucleotide identity with the PFRV reference strain (Genogroup III). The similarity between another PFRV-like virus isolated from grass carp and representatives of Genogroups III and IV was also <80%, indicating that this virus belonged to a third group (Genogroup II). All of the putative SVC viruses were assigned to a 4th group (Genogroup I), sharing <61% nucleotide identity with viruses in Genogroups II to IV.

KEY WORDS: Rhabdovirus · PFRV · SVCV · Sequence analysis · Genogroups

INTRODUCTION

Pike fry rhabdovirus (PFRV) was isolated from moribund pike fry *Exos lucius* during an outbreak of 'red disease' in a Dutch pike hatchery in 1973 (de Kinkelin et al. 1973). There have been few additional reports of PFRV isolation from pike. However, antigenically related viruses have been isolated from grass carp *Ctenopharyngodon idella* (Ahne 1975), tench *Tinca tinca*, white bream *Blicca bjoerkna* (Ahne et al. 1982), false harlequin *Pseudorasbora parva* (Ahne & Thomsen 1986), roach *Rutilus rutilus* (Haenen & Davidse 1989), sheatfish *Siluris glanis* (Fijan et al. 1984) and orfe *Leuciscus idus* (Dixon et al. 1994). Spring viraemia of carp (SVC) virus, a closely related rhabdovirus, causes a severe haemorrhagic rhabdovirus disease of cyrinids and is notifiable to the Office International des Epizooties (OIE), but PFRV was not placed on the notifiable list on the grounds that it was not considered to be a serious disease problem.

In 1998 a PFRV-like virus was isolated following mortalities in wild bream *Abramis brama* stocks in the River Bann, Northern Ireland (Rowley et al. 2001), having been isolated from brown trout *Salmo trutta* from the same country in a previous year (Adair & McLoughlin 1986). The virus reacted strongly in the ELISA using antisera raised against SVCV and PFRV but was tentatively identified as PFRV on the basis of a serum neutralisation assay. Sequence analysis showed, however, that the bream isolate shared <67.4 and <82.4% nucleotide identity with SVCV and PFRV respectively, and suggested that these viruses may belong to a third distinct genogroup of fish vesiculoviruses (Rowley et al. 2001).

It is widely recognised that SVCV cannot always be readily distinguished from PFRV using the cross-neutralisation assay and ELISA-based methods recom-

*Email: d.m.stone@cefas.co.uk

mended by the OIE, raising concerns about the reliability of some previous SVCV and PFRV diagnoses. SVCV was distinguished from PFRV isolates using an SVCV-based ribonuclease protection assay (Ahne et al. 1998), and some genomic differences were identified between the SVCV strains tested. However, due to a lack of PFRV sequence at the time of the study, it was not possible to perform reciprocal experiments, and the extent of any variation between different 'PFRV-like' viruses was not determined. Given the current legislation governing SVCV isolation as compared to PFRV, and the possible emergence of a third PFRV-like group of viruses infecting both salmonid and cyprinid species (Adair & McLoughlin 1986, Rowley et al. 2001), it is important that the fish rhabdoviral isolates can be identified accurately and reliably, particularly in those situations where there is some uncertainty because of cross-reactivity using serological techniques.

The utility of RT-PCR in the identification of fish rhabdoviruses and its greater ability to discriminate between isolates is already documented (Benmansour et al. 1997, Stone et al. 1997, Snow et al. 1999, Rowley et al. 2001). This paper describes an initial study undertaken to determine the genetic relatedness of SVCV and PFRV. A 550 nt region of the glycoprotein gene has been sequenced for 36 putative SVCV and PFRV isolates from a wide range of geographical locations and from different fish species, including several PFRV-like isolates that cross-react in both the ELISA and immunofluorescent antibody tests (IFAT) using anti-serum raised against SVCV.

MATERIALS AND METHODS

Potential SVCV primer annealing sites were identified by the alignment of the published amino acid sequences for the glycoprotein of SVCV (Bjorklund et al. 1996; GenBank accession no. U18101), the vesicular stomatitis New Jersey virus (VSNJV) (Gallione & Rose 1983; GenBank accession no. V01214), and the Piry virus (GenBank accession no. D26175). Primers were then designed to anneal to the regions encoding the conserved amino acids using the published sequence for SVCV (Björklund et al. 1996) as a skeleton, and introducing degenerate bases at the 3′ termini to allow for potential differences in codon usage (Fig. 1). The rationale for this approach to primer design was that if the amino acids are conserved between 3 distinct viruses, it is highly likely they have a functional role and would be highly conserved between viruses of the same group. Viral RNA was extracted from 100 µl of viral supernatant from infected EPC-cells using the Trizol Reagent™ (BRL, Life Technologies) and a protocol described previously (Strømmen & Stone 1997). Reverse transcription (RT) and the first round of PCR were performed using Ready-to-Go tubes (Pharmacia Biotech). RT was performed at 37°C for 1h in a 33 µl volume consisting of 50 mM Tris (pH 8.3), 75 mM KCL, 7.5 mM DTT, 10 mM $MgCl_2$, 0.08 mg ml^{-1} RNase and DNase free BSA, 10 mM dNTP, 500 ng SVCV R2 primer, M-MLV reverse transcriptase (20 U) and $^1/_{10}$ of the total RNA extracted above. The first round of PCR was performed in a 100 µl reaction volume containing the 33 µl RT reaction mix, 500 ng of the SVCV F1 primer and 2.5 U of Taq polymerase (BRL, Life Technologies). The reaction mix was overlaid with mineral oil and subjected to 30 temperature cycles of 1 min at 95°C, 1 min at 55°C and 1 min at 72°C followed by a final extension step of 10 min at 72°C.

Where products were not generated using a single round PCR, a second round of amplification was performed. This was performed using 5 µl of the first

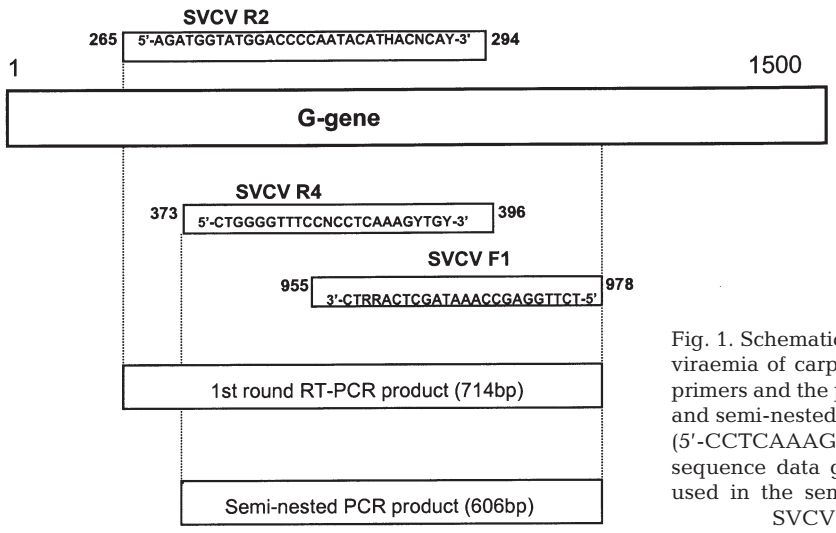

Fig. 1. Schematic diagram showing the location of the spring viraemia of carp virus (SVCV)-specific reverse and forward primers and the product sizes expected after the single round and semi-nested PCR reactions. A modified SVCV R4 primer (5′-CCTCAAAGYTGYGGNTGGGC-3′) was designed using sequence data generated for virus isolate 950237 and was used in the semi-nested PCR in situations where primers SVCV R4/F1 failed to generate a product

round of amplification in a 100 µl reaction volume containing 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, and 0.1% Triton X-100), 2.5 mM $MgCl_2$, 1.0 mM dNTPs, 500 ng of the SVCV R4 and SVCV F1 primers and 2.5 U of *Taq* polymerase. Reaction mixes were overlaid with mineral oil and subjected to 30 amplification cycles as before.

Aliquots (20 µl) of the amplified products were electrophoresed in a 2% (w/v) agarose/TAE (40 mM Tris-acetate, pH 7.2, 1 mM EDTA) gel containing 1.0 µg ml^{-1} ethidium bromide, and visualised by UV irradiation. PCR products were purified using the Geneclean® (BIO 101) and ligated into the pGEM-T vector using the standard protocol. Both DNA strands were sequenced using the M13 universal sequencing primers and the ABI PRISM™ dye terminator cycle sequencing system (Perkin Elmer). Sequencing reactions were analysed on an ABI 310 genetic analyser.

Table 1. Rhabdovirus isolates included in the studies. SVCV: Spring viraemia of carp virus pike fry rhabdoviruses; PFRV: pike fry rhabdoviruses. Initial identification was based on ELISA, serum neutralisation or immuno-fluorescence tests. Genogroups were assigned as part of this study

Isolate identifier	Host name	Year of isolation	Country of isolation	Initial identification	Geno-group
2/90	Common carp (*Cyprinus carpio*)	1990	Moldova	SVCV	Ib
332	White bream (*Blicca bjoerkna*)	1981	Germany (Ahne et al. 1982)	PFRV	IV
770346	Common carp	1977	UK	SVCV	Id
80560	Roach (*Rutilus rutilus*)	1983	The Netherlands (Haenen & Davidse 1989, 1993)	PFRV	IV
84/4	Brown trout (*Salmo trutta*)	1984	UK (N. Ireland) (Adair & McLoughlin 1986)	PFRV	IV
860115	Common carp	1986	UK	SVCV	Id
880062	Common carp	1988	UK	SVCV	Id
880124	Common carp	1988	UK	SVCV	Id
880137	Orfe or ide (*Leuciscus idus*)	1988	UK	PFRV	IV
950237	Tench (*Tinca tinca*)	1995	UK	PFRV	IV
970395	Common carp	1997	UK	SVCV	Id
970469	Common carp	1997	UK	SVCV	Ia
980624	Orfe	1998	UK	PFRV	IV
98-109	Bream	1998	UK (N. Ireland) (Rowley et al. 2001)	PFRV	IV
980451	Koi carp (*Cyprinus carpio*)	1998	UK[a]	SVCV	Ia
980528 1.1	Koi carp	1998	UK[a]	SVCV	Ia
98-93	Brown trout	1998	UK (N. Ireland) (Rowley et al. 2001)	PFRV	IV
993890 2.1	Bream (*Abramis brama*)	1999	UK	PFRV	IV
993890 7.1	Grass carp (*Ctenopharyngodon idella*)	1999	UK	PFRV	IV
993890 9.1	Bream	1999	UK	PFRV	IV
993890 11.1	Roach	1999	UK	PFRV	IV
994591	Tench	1999	UK	PFRV	IV
994602 3.1	Crucian carp (*Carassius carassius*)	1999	UK	PFRV	IV
994663 1.1	Bream	1999	UK	PFRV	IV
994663 2.1	Roach	1999	UK	PFRV	IV
994668 1.1	Bream	1999	UK	PFRV	IV
M2-78	Silver carp (*Hypophthalmichthys molitrix*)	1983	Moldova (Shchelkunov & Shchelkunova 1989)	SVCV	Id
N1-5	Bighead carp (*Aristichthys nobilis*)	1986	Ukraine	SVCV	Ic
N3-14	Grass carp	1986	Ukraine	SVCV	Id
P4	Common carp	1983	Russia	SVCV	Ib
PFRV F4	Pike (*Exos lucius*)	1973	France (de Kinkelin et al. 1973)	PFRV	III
PPRV	False harlequin (*Pseudorasbora parva*)	1986	Germany (Ahne & Thomsen 1986)	PFRV	IV
RHV	Rainbow trout (*Oncorhynchus mykiss*)	1989	Ukraine	SVCV	Ic
S 30	Common carp	1971	Yugoslavia (Fijan et al. 1971)	SVCV	Id
S 64	Tench	1982	Germany (Ahne et al. 1982)	PFRV	IV
V 76	Grass carp	1982	Germany (Ahne 1975)	PFRV	II

[a]Viruses were isolated during routine import checks on fish originating from China

Phylogenetic analysis was performed using PHYLIP (Felsenstein 1989) within the phylogenetic inference environment (PIE) at the Human Genome Mapping Project Resource Centre (HGMP-RC), Hinxton, UK. Estimations of the phylogenies were done using 1000 data sets that had been randomly resampled from the original data using the bootstrap method. Phylogenetic trees were generated by maximum parsimony and Neighbor-Joining analysis of 36 partial G-gene sequences from putative SVCV and PFRV isolates, and the corresponding G-gene sequence from VSV Piry strain (GenBank accession no. D26175) was used as an outgroup.

The rhabdovirus isolates included in this study, their origins and the initial identification are listed in Table 1. Amplification products were generated for all viruses previously confirmed as SVCV by the serological tests. However, it was necessary in most cases where the virus had previously been identified as PFRV to perform a second round of PCR. Further, when amplifying the PFRV reference strain, F4, and the isolate from grass carp, V76, the modified SVCV R4 primer was required (Fig. 1). At least 2 independent cloning events were performed for each virus isolate to eliminate errors introduced by the *Taq* polymerase and to determine the consensus sequence within what was likely to be a complex heterologous 'quasi-species'.

RESULTS

Maximum parsimony analysis of the partial G-gene sequences identified 4 genogroups that were supported by bootstrap values in excess of 974 (Fig. 2). No

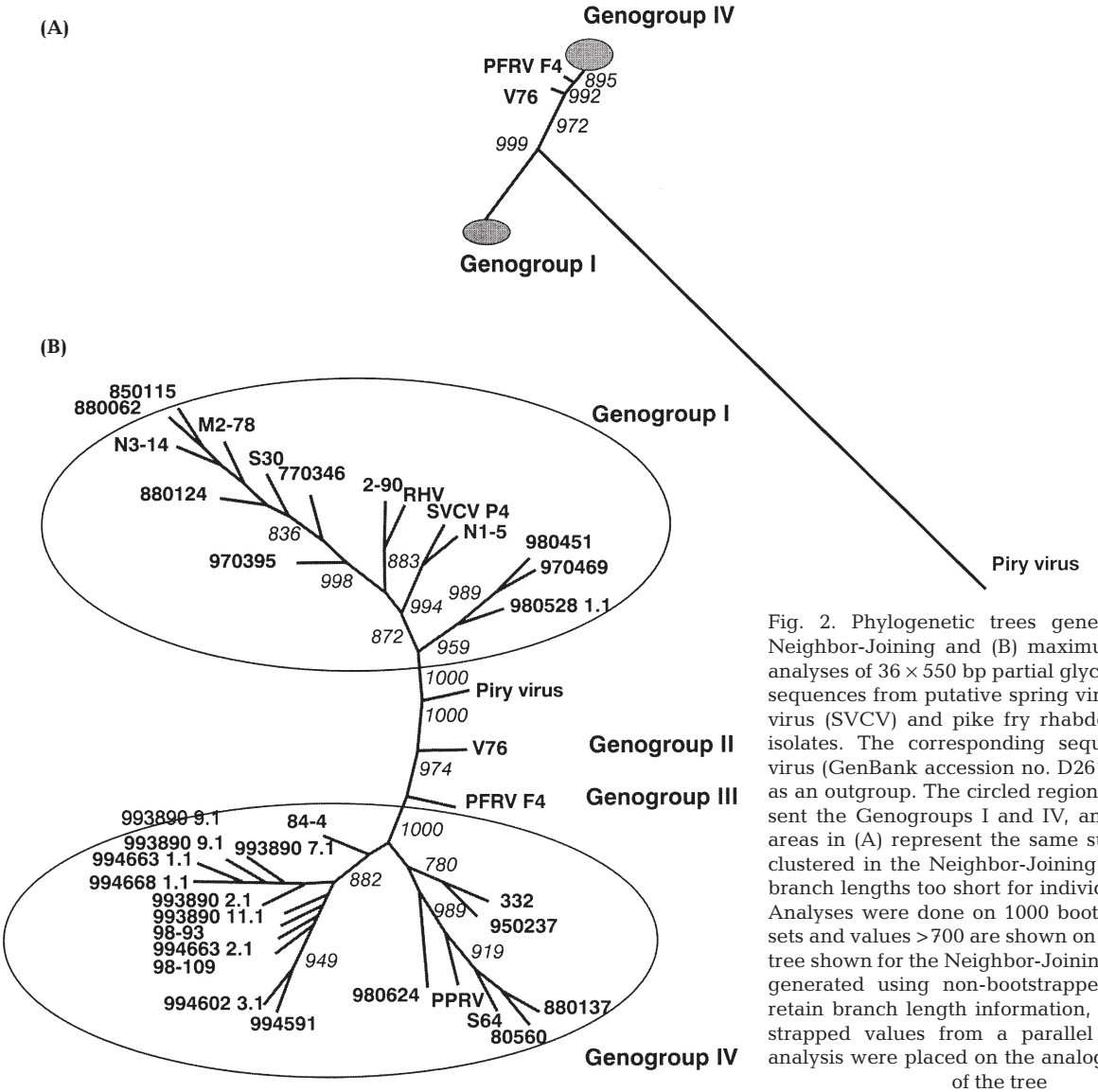

Fig. 2. Phylogenetic trees generated by (A) Neighbor-Joining and (B) maximum parsimony analyses of 36 × 550 bp partial glycoprotein gene sequences from putative spring viraemia of carp virus (SVCV) and pike fry rhabdovirus (PFRV) isolates. The corresponding sequence of Piry virus (GenBank accession no. D26175) was used as an outgroup. The circled regions in (B) represent the Genogroups I and IV, and the shaded areas in (A) represent the same subgroups that clustered in the Neighbor-Joining analysis with branch lengths too short for individual labelling. Analyses were done on 1000 bootstrapped data sets and values >700 are shown on the trees. The tree shown for the Neighbor-Joining method was generated using non-bootstrapped analysis to retain branch length information, and the bootstrapped values from a parallel bootstrapped analysis were placed on the analogous branches of the tree

correlation was observed between the genogroups and the host species. The viruses assigned to Genogroup I were primarily isolated from common carp; however, viruses assigned to the same group were also isolated from silver carp, bighead carp and grass carp, and from rainbow trout. Viruses assigned to Genogroup IV were isolated from a wide range of cyprinids including the common carp, and from brown trout. Only single virus isolations were assigned to the Genogroups II and III.

Those viruses previously identified as SVCV based on serological data were assigned to Genogroup I. These viruses showed a higher-than-expected level of nucleotide sequence diversity (82.7 to 100% identity) indicating that SVCV has probably evolved independently in several different geographical areas. Indeed, the branch separating viruses originating in the Republic of China (isolates 980451 and 980528 1.1) from those viruses originating in Moldova, the Ukraine, Russia and the UK was supported by a bootstrap value of 959. Isolate 970469, which also clustered with 980451 and 980528, was recovered from fish held on a site that had received several consignments of fish from the Republic of China. Re-analysis of the sequence data generated for viruses assigned to Genogroup I only (Fig. 3) identified 4 subgroups (Ia–d) supported by bootstrap values of >985. Those viruses originating in Asia were assigned to Subgroup Ia, those from Moldova, the Ukraine and Russia to Subgroups Ib and Ic, and those from the UK to Subgroup Id. Some isolates from the states of the former USSR (M2-78 and N3-14) also clustered with the SVC viruses traditionally associated with SVC in Europe. The reason for this remains unclear and warrants further investigation.

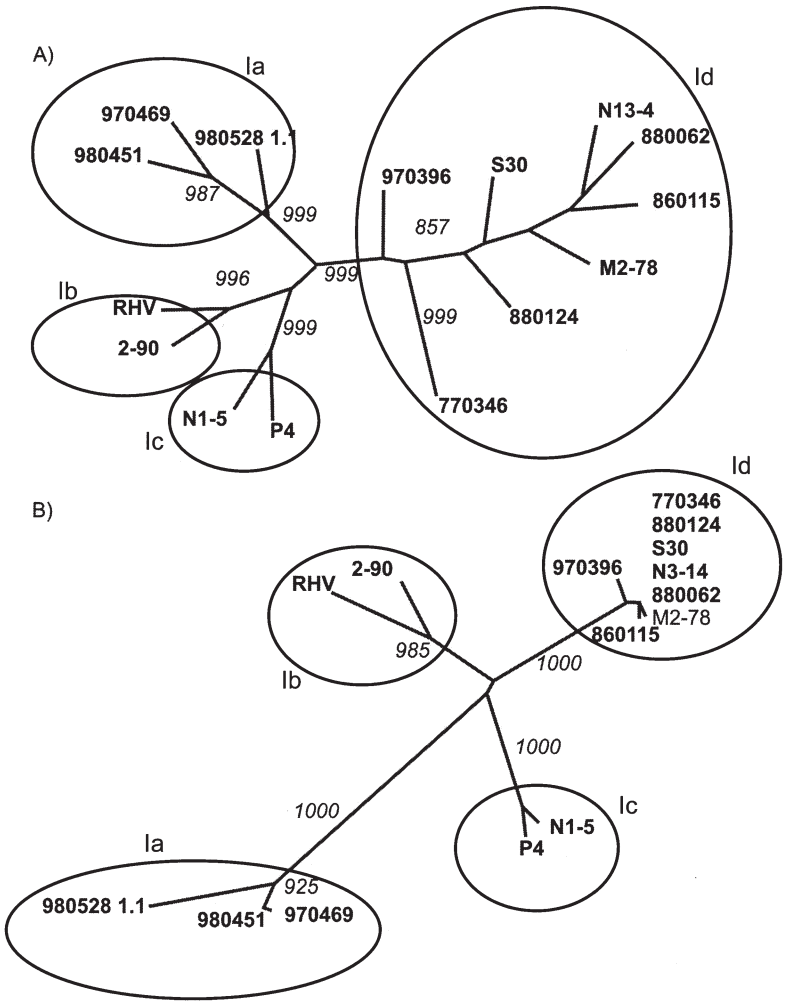

Fig. 3 Phylogenetic trees generated by (A) maximum parsimony and (B) Neighbor-Joining analyses of 550 bp partial glycoprotein gene sequences from 15 spring viraemia of carp virus (SVCV) isolates. Analyses were done on 1000 bootstrapped data sets and values >700 are shown on the trees. The tree shown for the Neighbor-Joining method was generated using non-bootstrapped analysis to retain branch length information, and the bootstrapped values from a parallel bootstrapped analysis were placed on the analogous branches of the tree

The majority of the remaining isolates (19 of 21), including many previously identified as PFRV (Vestergård Jorgensen et al. 1989), were assigned to Genogroup IV. These isolates showed a high degree of nucleotide sequence identity to each other (>93.7%). However, when compared to the PFRV reference strain F4 they shared <80% nucleotide sequence identity and were considered sufficiently different for PFRV to be assigned to a separate group (Genogroup III). A grass carp isolate (V76), which was also previously identified as PFRV, shared only <70% nucleotide sequence identity with both PFRV F4 and representatives of Genogroup IV and was assigned to Genogroup II. Phylogenetic trees generated using the deduced amino acid sequences identified the same 4 genogroups with only minor changes in the branching orders (data not shown). The relatively high degree of amino acid sequence identity observed between viruses from Genogroups I–IV (Fig. 4) could explain the significant cross-reactivity observed between representatives of Genogroups I and IV in ELISA when using polyclonal anti-serum raised against SVCV (Rowley et al. 2001).

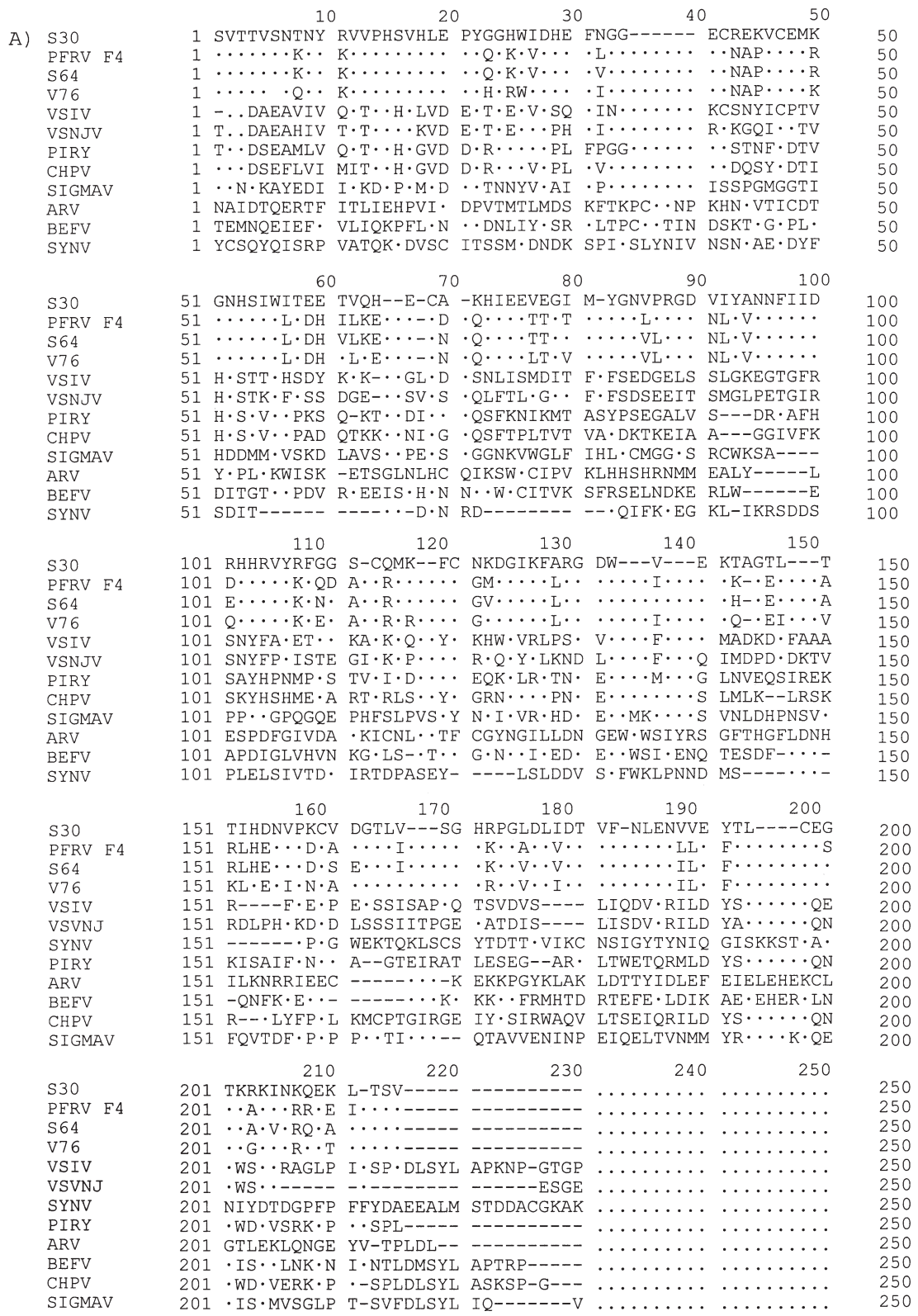

Fig. 4. (Above and facing page.) (A) Alignment of the deduced amino acids (Residues 136 to 318) of the glycoprotein gene of the spring viraemia of carp virus (SVCV) reference strain (S30), the pike fry rhabdoviruses (PFRV) reference strain (F4), 2 putative PFRV isolates (S64 and V76), with the equivalent region of the glycoprotein gene of a range of rhabdoviruses of terrestrial animals (VSIV, VSNJV, PIRYV, CHPV, ARV and BEFV), insects (SIGMAV) and plants (SYNV). (**.**) Positions of sequence identity compared to the sequence for the SVCV reference strain. (–) Gaps introduced to improve the alignment

B)

	S30	PFRV F4	S64	V76	VSIV	VSNJV	PIRY	CHPV	SIGMAV	ARV	BEFV	SYNV
S30		64.6	56.3	70.3	17.1	22.2	22.4	18.4	12.7	15.8	15.8	10.8
PFRV F4			90.3	80.0	23.2	20.5	21.6	22.8	16.2	15.1	15.7	9.2
S64				81.2	22.4	20.6	20.6	23.6	16.4	15.2	14.5	8.5
V76					21.1	23.2	22.7	22.2	16.8	13.0	16.2	10.3
VSIV						45.7	32.4	31.6	23.0	14.8	16.3	10.5
VSNJV							33.3	30.1	15.6	14.5	14.0	10.8
PIRY								48.4	19.1	13.8	13.3	10.6
CHPV									18.5	19.5	16.5	11.1
SIGMAV										15.0	16.0	10.0
ARV											32.5	10.5
BEFV												9.5
SYNV												

Fig. 4. (Continued). (B) Deduced amino acid sequence identity matrix for the viruses given in (A)

DISCUSSION

When comparing the equivalent region of the glycoprotein from a number of rhabdoviruses, including vesiculoviruses infecting terrestrial animals, the same high degree of amino acid sequence identity was not observed (Fig. 4). The data are not consistent with the observations of Björklund et al. (1996), which showed a relatively high degree of amino acid sequence identity between SVCV and vesicular stomatitis New Jersey virus, and probably reflects the use of a more variable region of the glycoprotein gene sequence in the current analysis. Importantly, however, it highlights that while some short amino acid motifs are well conserved between VSV and SVCV, the region of the glycoprotein under study here is highly conserved at the amino acid level between the SVCV/PFRV Genogroups I to IV but shares little homology with other vesiculovirus species. Currently SVCV and PFRV are only tentatively recognised as vesiculoviruses by the International Committee on Taxonomy of Viruses (ICTV) (Van Regenmortel et al. 2000). The data presented here, together with the observation that SVCV, PFRV and the related viruses only infect aquatic animals, suggest that in the future it may be more appropriate for these viruses to be assigned to a separate aquatic rhabdovirus genus. A comprehensive sequencing program is currently underway to establish if the aquatic vesiculotype viruses, including those described by others (Dorson et al. 1984, Johansson et al. 2001) exhibit the relevant properties to be formally recognised as species within the vesiculovirus genus, or if they have sufficient unique properties to place them in a separate genus within the family *Rhabdoviridae.* Other characteristics such as natural host range, cell and tissue tropism, and physiological and antigenic properties will also be investigated.

The results presented here further highlight the utility of RT-PCR-based methods in the identification of fish rhabdoviruses and its greater ability to discriminate between serologically related viruses. The sequence differences observed in the glycoprotein gene of SVCV isolates from different geographical areas, particularly those originating in Asia, also highlight the value that this region of the genome could have in future epidemiological investigations.

Acknowledgements. A.M.S. and C.T.-Y.L. were industrial placement students sponsored by the Department for Environment, Food and Rural Affairs (Defra), contract numbers CSA3114 and CSA3342. The work was conducted under Defra contracts FC1105 and F1118, and CEFAS seedcorn contract DP105. We would like to thank B. M. Adair, P. de Kinkelin, O. L. M. Haenen, and I. S. Shchelkunov for kindly providing their virus isolates. The EMBL accession numbers for the sequences reported in this study are AJ 538060–AJ538095.

LITERATURE CITED

Adair BM, McLoughlin M (1986) Isolation of pike fry rhabdovirus from brown trout (*Salmo trutta*). Bull Eur Assoc Fish Pathol 6:85–86

Ahne W (1975) A rhabdovirus isolated from grass carp (*Ctenopharyngodon idella* Val.). Arch Virol 48:181–185

Ahne W, Thomsen I (1986) Isolation of pike fry rhabdovirus from *Pseudorasbora parva* (Temminck & Schlegel). J Fish Dis 9:555–556

Ahne W, Mahnel H, Steinhagen P (1982) Isolation of pike fry rhabdovirus from tench, *Tinca tinca* L., and white bream, *Blicca bjoerkna* (L.). J Fish Dis 5:535–537

Ahne W, Kurath G, Winton JR (1998) A ribonuclease protection assay can distinguish SVCV from PFR. Bull Eur Assoc Fish Pathol 6:220–224

Benmansour A, Bernardo B, Monnier AF, Vende P, Winton JR, de Kinkelin P (1997) Sequence variation of the glycoprotein gene identified three distinct lineages within field isolates of viral haemorrhagic septicaemia, a fish rhabdovirus. J Gen Virol 78:2837–3846

Björklund HV, Higman KH, Kurath G (1996) The glycoprotein genes and gene junctions of the fish rhabdoviruses, spring viremia of carp virus and hirame rhabdovirus: analysis of relationships with other rhabdoviruses. Virus Res 42: 65–80

de Kinkelin P, Galimard B, Bootsma R (1973) Isolation and identification of the causative agent of 'red disease' of pike (*Esox lucius* L. 1766). Nature 241:465–467

Dixon PF, Hattenberger-Baudouy AM, Way K (1994) Detection of carp antibodies to spring viraemia of carp virus by competitive immunoassay. Dis Aquat Org 19:181–186

Dorson M, Torchy C, Chilmonczyk S, de Kinkelin P, Michel C (1984) A rhabdovirus pathogenic for perch, *Perca fluviatilis* L.: isolation and preliminary study. J Fish Dis 7: 241–245

Felsenstein (1989) Phylip: phylogeny inference package (version 3.5). Cladistics 5:164–166

Fijan N, Petrinec Z, Sulimanovic D, Zwillenberg LO (1971) Isolation of the viral causative agent from the acute form of infectious dropsy of carp. Vet Arch 41:125–138

Fijan N, Matasin Z, Jeney Z, Oláh J, Zwillenberg LO (1984) Isolation of Rhabdovirus carpio from sheatfish (*Silurus glanis*) fry. In: Olah J (ed) Fish, pathogens and environment in European polyculture. Akadémiai Kiadó, Budapest, p 17–24

Gallione CJ, Rose, JK (1983) Nucleotide sequence of a clone encoding the entire glycoprotein from the New Jersey serotype of vesicular stomatitis virus. J Virol 46:162–169

Haenen OLM, Davidse A (1989) Isolation of pike fry rhabdovirus from roach (*Rutilus rutilus*). Bull Eur Assoc Fish Pathol 9:116

Haenen OLM, Davidse A (1993) Comparative pathogenicity of two strains of pike fry rhabdovirus and spring viremia of carp virus for young roach, common carp, grass carp and rainbow trout. Dis Aquat Org 15:87–92

Johansson T, Nylund S, Olesen NJ, Björklund (2001) Molecular characterisation of the nucleocapsid protein gene, glycoprotein gene and gene junctions of rhabdovirus 903/87, a novel fish pathogenic rhabdovirus. Virus Res 80:11–22

Rowley H, Graham DA, Campbell S, Way K, Stone DM, Curran WL, Bryson DG (2001) Isolation and characterisation of rhabdovirus from wild common bream *Abramis brama*, roach *Ritilus rutilus*, farmed brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* in Northern Ireland. Dis Aquat Org 48:7–15

Shchelkunov IS, Shchelkunova TI (1989) Rhabdovirus carpio in herbivorous fishes: isolation, pathology and comparative susceptibility of fishes. In: Ahne W, Kurstak E (eds) Viruses of lower vertebrates. Springer-Verlag, Berlin, p 333–348

Snow M, Cunningham CO, Melvin WT, Kurath G (1999) Analysis of the nucleoprotein gene identifies distinct lineages of viral haemorrhagic septicaemia virus within the European marine environment. Virus Res 63:35–44

Stone DM, Way K, Dixon PF (1997) Nucleotide sequence of the glycoprotein gene of viral haemorrhagic septicaemia (VHS) viruses from different geographical areas: a link between VHS in farmed fish species and viruses isolated from North Sea cod (*Gadus morhua* L.). J Gen Virol 78: 1319–1326

Strømmen HK, Stone DM (1997) Detection of viral haemorrhagic septicaemia (VHS) virus in fish tissues by seminested polymerase chain reaction (PCR). In: Barnes A, Davidson G, Hiney M, McIntosh D (eds) Proc Symp Methodology in Fish Disease Research. Fisheries Research Services, Aberdeen, p 203–209

Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB and 7 others (2000) Family Rhabdoviridae. In: Virus taxonomy. Seventh Rep Int Comm Taxonomy of Viruses. Academic Press, London, p 563–583

Vestergård Jorgensen PE, Olesen NJ, Ahne W, Lorenzen N (1989) SVCV and PFR viruses: serological examination of 22 isolates indicates close relationship between the two fish rhabdoviruses. In: Ahne W, Kurstak E (eds) Viruses of lower vertebrates. Springer-Verlag, Berlin, p 349–366

Editorial responsibility: Jo-Ann Leong, Kaneohe, Hawaii, USA

Submitted: March 5, 2002; Accepted: November 1, 2002
Proofs received from author(s): February 7, 2003