Template for Taxonomic Proposal to the ICTV Executive Committee
Creating Species in an existing genus

Code[†] | 2007.138V.04 | To designate the following as species in the genus:

Aquareovirus G

belonging to the family° : *Reoviridae*

Aquareovirus G
> **American grass carp reovirus**
> **Golden Ide reovirus**

[†] Assigned by ICTV officers
° leave blank if inappropriate or in the case of an unassigned genus

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Old Taxonomic Order

Order
Family   *Reoviridae*
Genus   *Aquareovirus*
Type Species   *Aquareovirus A*
Species in the genus

Aquareovirus A **(AQRV-A)**
American oyster reovirus 13p2V	(13p2V)
Angel fish reovirus	(AFRV)
Atlantic salmon reovirus HBR	(HBRV)
Atlantic salmon reovirus ASV	(ASRV)
Atlantic salmon reovirus TSV	(TSRV)
Chinook salmon reovirus DRC	(DRCRV)
Chum salmon reovirus CSV	(CHSRV)
Masou salmon reovirus MS	(MSRV)
Smelt reovirus	(SRV)
Striped bass reovirus	(SBRV)

Aquareovirus B **(AQRV-B)**
Chinook salmon reovirus B	(GRCV)
Chinook salmon reovirus LBS	(LBSV)
Chinook salmon reovirus YRC	(YRCV)
Chinook salmon reovirus ICR	(ICRV)

Coho salmon reovirus CSR	(CSRV)
Coho salmon reovirus ELC	(ELCV)
Coho salmon reovirus SCS	(CSCV)

Aquareovirus C (**AQRV-C**)

Golden shiner reovirus	(GSRV)
Grass carp reovirus	(GCRV)

Aquareovirus D (**AQRV-D**)

Channel catfish reovirus	(CCRV)

Aquareovirus E (**AQRV-E**)

Turbot reovirus	(TRV)

Aquareovirus F (**AQRV-F**)

Chum salmon reovirus PSR	(PSRV)
Coho salmon reovirus SSR	(SSRV)

Tentative Species in the Genus

Chub reovirus Germany	(CHRV)
Hard clam reovirus	(HCRV)
Landlocked salmon reovirus	(LSRV)
Tench reovirus	(TNRV)
Golden ide reovirus	(GIRV)

Unassigned Species in the Family

New Taxonomic Order

Order	
Family	*Reoviridae*
Genus	*Aquareovirus*
Type Species	*Aquareovirus A*

Species in the genus

Aquareovirus A (**AQRV-A**)

American oyster reovirus 13p2V	(13p2V)
Angel fish reovirus	(AFRV)
Atlantic salmon reovirus HBR	(HBRV)
Atlantic salmon reovirus ASV	(ASRV)
Atlantic salmon reovirus TSV	(TSRV)
Chinook salmon reovirus DRC	(DRCRV)
Chum salmon reovirus CSV	(CHSRV)
Masou salmon reovirus MS	(MSRV)
Smelt reovirus	(SRV)
Striped bass reovirus	(SBRV)

Aquareovirus B (**AQRV-B**)

Chinook salmon reovirus B	(GRCV)
Chinook salmon reovirus LBS	(LBSV)
Chinook salmon reovirus YRC	(YRCV)
Chinook salmon reovirus ICR	(ICRV)
Coho salmon reovirus CSR	(CSRV)
Coho salmon reovirus ELC	(ELCV)
Coho salmon reovirus SCS	(CSCV)

Aquareovirus C (**AQRV-C**)
Golden shiner reovirus	(GSRV)
Grass carp reovirus	(GCRV)

Aquareovirus D (**AQRV-D**)
Channel catfish reovirus	(CCRV)

Aquareovirus E (**AQRV-E**)
Turbot reovirus	(TRV)

Aquareovirus F (**AQRV-F**)
Chum salmon reovirus PSR	(PSRV)
Coho salmon reovirus SSR	(SSRV)

Aquareovirus G (**AQRV-F**)
American grass carp reovirus	(AGCRV)
Golden ide reovirus	(GIRV)

Tentative Species in the Genus
Chub reovirus Germany	(CHRV)
Hard clam reovirus	(HCRV)
Landlocked salmon reovirus	(LSRV)
Tench reovirus	(TNRV)

Unassigned Species in the Family

ICTV-EC comments and response of the SG

Species demarcation criteria in the genus

Within the family *Reoviridae*, the prime determinant for inclusion of virus isolates within a single virus species is their ability to exchange genetic information during co-infection, by genome segment reassortment, thereby generating viable progeny virus strains. However, data providing direct evidence of segment reassortment between isolates of aquareoviruses are not available. RNA cross-hybridization studies and serological comparisons are therefore the methods most commonly used to examine the level of similarity that exists between isolates. The methods which form the "species parameters" can in effect be used to predict the compatibility of related viruses for genome segment reassortment.

Members of a single aquareovirus species may be identified by:
- Their ability to exchange genetic material by genome segment reassortment during dual infections, thereby producing viable progeny virus strains.
- Cross-hybridization assays (Northern or dot blot), with probes made from viral RNA or cDNA. For example, in Northern hybridization assays, conditions (stringency) that do not allow <17% mismatch will not show any hybridization between viruses from two different species, while viruses within a species will show hybridization.
- RNA sequence analysis (viruses within different species should have low levels of sequence homology among the cognate genome segments). For example, genome segment 10 that encodes the major outer CP, VP7, will show > 45% sequence variation between viruses from two different species. These nucleotide sequence differences should also be reflected in the aa sequence variation (> 64%) of the VP7 proteins.
- Serological comparisons of antigens or antibodies by neutralization (or other) assays using, either polyclonal antisera or monoclonal antibodies against conserved antigens. For example, cross-neutralization assays, using polyclonal rabbit antisera separate different aquareoviruses into the same groupings as the cross-hybridization assays.
- Analysis of "electropherotype" by agarose gel electrophoresis (AGE). For example, viruses within *Aquareovirus-A* will show a relatively uniform electropherotype, while viruses belonging to *Aquareovirus-B* will show a different, but also relatively uniform, electropherotype. However, similarities in migration rates of some genome segments can exist between species.
- Identification of the conserved terminal regions of the genome segments (Some closely related species can also have identical terminal sequences on at least some segments).

Six species (*Aquareovirus-A* to *Aquareovirus-F*) and some unassigned viruses have been recognized on the basis of RNA-RNA hybridization

Argumentation to justify the designation of new species in the genus

American grass carp reovirus (AGCRV) has been shown to possess an eleven-segmented dsRNA genome and was isolated from diseased and asymptomatic fishes. The virus was incriminated in the death of fingerling grass carp and fathead minnow fishes. The virus was found to replicate in fish cells, particularly the FHM cells and is an example of non-syncytialising aquareoviruses (Mohd Jaafar *et al.*, submitted). The full-length genome was cloned and sequenced completely (Mohd Jaafar *et al.*, submitted).

The conserved terminal regions of the AGCRV genome segments are 5'-GUUUUA$^{U}/_{a}$----------------$^{U}/_{a}$$^{U}/_{a}$UCAUC-3'. These are similar but not identical to aquareovirus A (AQRV-A) and AQRV-C conserved termini.

Chum salmon reovirus (AQRV-A)	5'-GUUUUA$^{U}/_{G}$------------------$^{A}/_{U}$UCAUC-3'
Grass carp reovirus (AQRV-C)	5'-GUUAUU$^{U}/_{G}$-------------------$^{A}/_{U}$UCAUC-3'
American grass carp reovirus (AQRV-G)	5'-GUUUUA$^{U}/_{a}$----------------$^{U}/_{a}$$^{U}/_{a}$UCAUC-3'

These data also indicate that although AGCRV is an aquareovirus, it does not belong to any of the other *Aquareovirus* species.

The genome sequence of AGCRV was compared to those of CHSRV (AQRV-A), GCRV and GSRV (AQRV-C). Overall amino acid identity values ranging from 22 to 76% were detected between individual pairs of proteins (Table 1).

However, when AGCRV was compared to the as yet unclassified golden ide reovirus (GIRV), much higher levels of amino acid (aa) identity were detected (98% with the viral polymerase and 92% with the NTPase, encoded by Seg-2 and Seg-5 of GIRV respectively). These values are consistent with GIRV and AGCRV belonging to the same AQRV species.

Within a single *Aquareovirus* species, the polymerase sequence showed over 95% aa identity. Comparison of AGCRV polymerase with those of aquareoviruses showed that aa identities varied between 57 and 74%. These values are compatible with the classification of AGCRV as a distinct species. A phylogenetic tree constructed with the polymerase sequences of various members of family *Reoviridae*, including the aquareoviruses is shown in figure 1. This tree shows AGCRV clustering within genus *Aquareovirus*, on a separate branch (together with GIRV) from other aquareviruses.

Genome segment 10 of the aquareoviruses encodes major outer capsid protein VP7. VP7 was compared from different isolates of striped bass reovirus, chum salmon reovirus and threadfin reovirus (AQRV-A), coho salmon reovirus (AQRV-B), Grass carp reovirus and golden shiner reovirus (AQRV-C) and American grass carp reovirus (AGCRV). The results showed 14 to 99.6% aa identity overall, with >79% within a single species. Amino acid identities of 79.2 to 86.6% were detected between *iso*lates of AQRV-A: 95.8-99.6% within AQRV-C: but only 14 to 16% between AQRV-A and C, and 18.1 to 18.5%, between AQRV-B and C. Comparisons of AGCRV with AQRV-A, B or C, gave identity values of only 15.9 to 18.1%, 17.2%, or 24.3 to 26.7% respectively, confirming other indications that AGCRV represents a distinct species. A phylogenetic tree constructed using the VP7 available sequences (figure 2) also showed that AGCRV clustered on a separate branch and should be identified as a distinct species from those of AQRV-A, AQRV-B and AQRV-C.

The comparison of the electropherotypes of AGCRV and GIRV to those of members of species *Aquareovirus D, Aquareovirus E and Aquareovirus F* showed that members of the proposed 'Aquareovirus G' are distinct.

Host range, together with terminal sequence, sequence comparisons and the electropherotype analysis are all consistent with classification of AGCRV within a new *Aquareovirus* species, designated "Aquareovirus G".

List of created Species in the genus
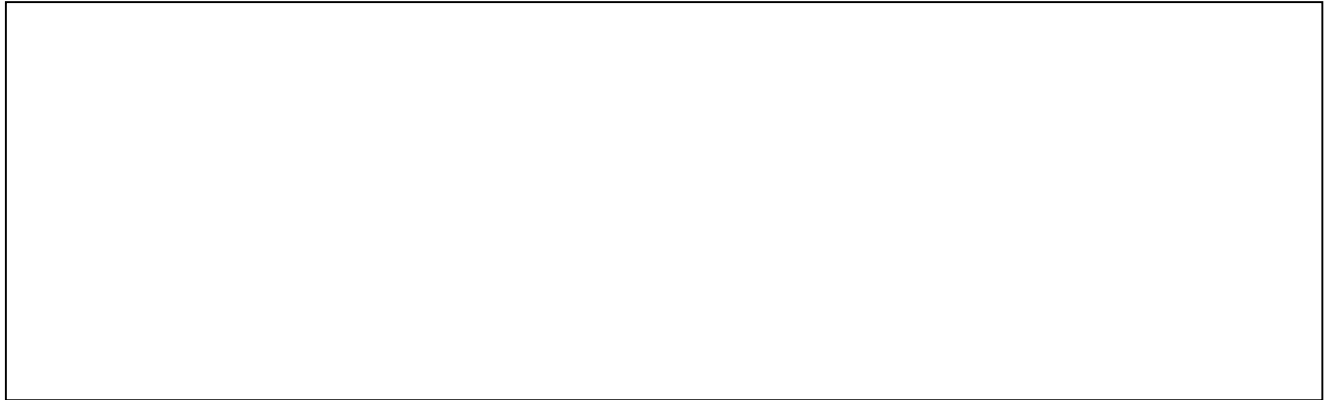
References

Fauziah MOHD JAAFAR, Andrew E. Goodwin, Mourad BELHOUCHET, Gwenn Merry, Qin FANG, Jean-François CANTALOUBE, Philippe BIAGINI, Philippe de MICCO, Peter P. C. MERTENS and, Houssam ATTOUI. Complete sequence analysis of American grass carp reovirus genome, a new member of the genus *Aquareovirus* (family *Reoviridae*): evidence for an evolutionary link between the aquareoviruses and coltiviruses. Submitted.

Mertens, P. P. C., <u>Attoui, H.</u>, Duncan, R. & Dermody, T. S. (2005). *Reoviridae*. In *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 447-454. Edited by C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger & L. A. Ball. London: Elsevier/Academic Press.

Attoui H, Fang Q, Mohd Jaafar F, Cantaloube JF, Biagini P, de Micco P, de Lamballerie X. (2002). Common evolutionary origin of aquareoviruses and orthoreoviruses revealed by genome characterization of Golden shiner reovirus, Grass carp reovirus, Striped bass reovirus and golden ide reovirus (genus Aquareovirus, family Reoviridae). *Journal of General Virology*. 83:1941-1951.
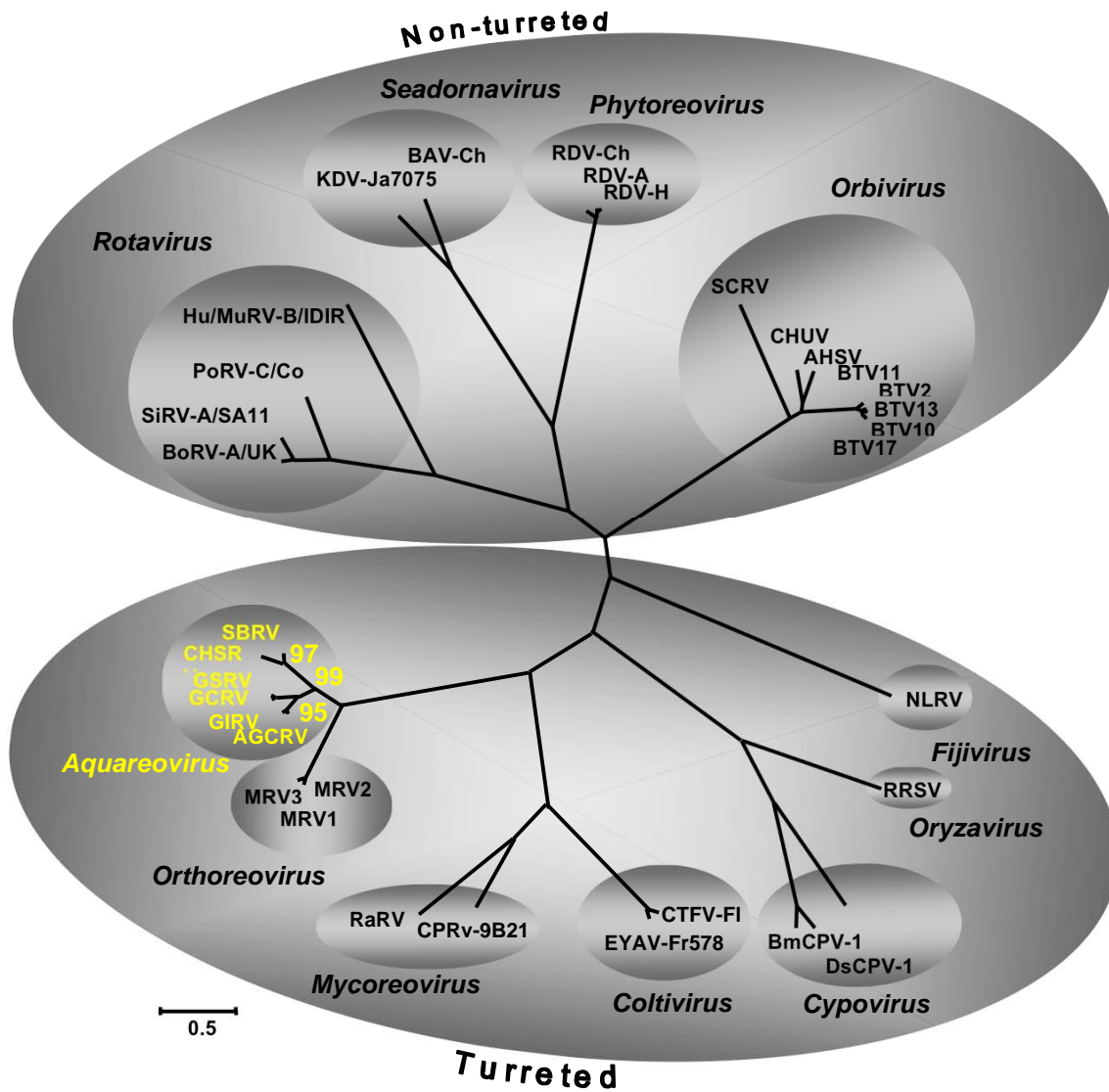
Annexes:

Figure 1 : Phylogenetic comparison of the viral polymerase VP1(Pol) proteins of AGCRV, other aquareovirus species and members of other genera within the family *Reoviridae*. The analysis (presented as radial tree) was constructed with the help of the MEGA3 programme using the p-distance algorithm. The cluster of aquareovirus is presented at the lower left of the tree and bootstrap values higher than 95% support the branching within this cluster. Accession numbers and further detail of the sequence and viruses used are from Attoui *et al*., 2001.
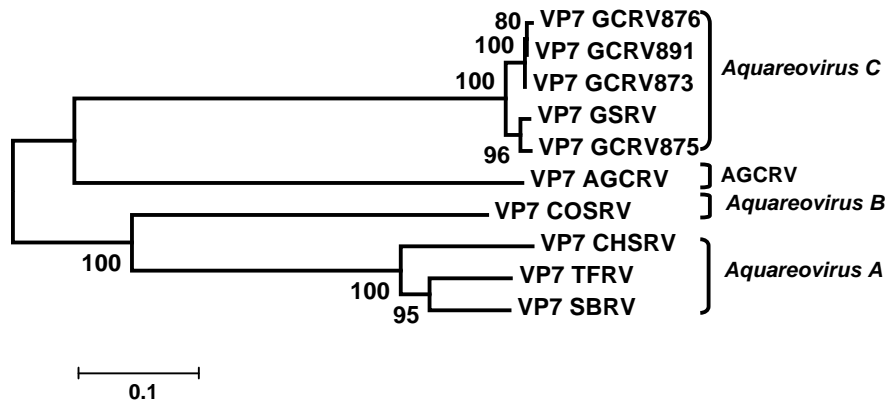
Figure 2: Neighbour-joining phylogenetic tree built with the major outer capsid protein VP7 (Seg-10) of aquareoviruses. The bootstrap values are shown at the nodes. The tree identifies AGCRV as a distinct species of aquareovirus.

Table 1: Comparison of segments 1 to 11 of aquareoviruses: calculation of nucleic acid and amino acid identities.

Virus	Segment	AGCRV Segments 1 to 11		CHSRV Segment 1 to 11		GCRV Segment 1 to 11	
		% NUC	% PROT	% NUC	% PROT	% NUC	% PROT
GSRV	1	62	63	54	44.	95	99
	2	68	74	64	65	94	99
	3	69	76	61	55	91	99
	4	54	41	51	26	96	99
	5	59	57	48	34	91	97
	6	66	68	58	50	94	99
	7	58	45 (NS31), 64 (NS16)	52	26 (NS31), 30 (NS16)	95	98 (NS31), 96 (NS16)
	8	63	61	56	45	95	99
	9	61	57	52	39	92	100
	10	53	30	49	20	92	96
	11	54	23	50	23	98	99
GCRV	1	62	63	54	44		
	2	68	74	64	65		
	3	69	76	62	55		
	4	53	41	51	26		
	5	59	57	47	34		
	6	67	69	57	50		
	7	56	45 (NS31), 63 (NS16)	52	26 (NS31), 30 (NS16)		
	8	63	61	55	45		
	9	91	57	53	39		
	10	54	31	49	20		
	11	54	24	50	24		
CHSRV	1	51	42				
	2	58	57				
	3	57	48				
	4	48	28				
	5	47	33				
	6	55	49				
	7	47	24 (NS31), 28 (NS16)				
	8	53	42				
	9	48	36				
	10	42	22				
	11	45	26				