



This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

<b>Code assigned:</b>	<b>2016.009a-kD</b>	(to be completed by ICTV officers)
<b>Short title:</b> Splitting family <i>Iridoviridae</i> into 2 new subfamilies; adding 1 new species (e.g. 6 new species in the genus <i>Zetavirus</i> )		
<b>Modules attached</b> (modules 1 and 11 are required)	2 <input checked="" type="checkbox"/> 3 <input type="checkbox"/> 4 <input checked="" type="checkbox"/> 5 <input type="checkbox"/> 6 <input type="checkbox"/> 7 <input type="checkbox"/> 8 <input type="checkbox"/> 9 <input type="checkbox"/> 10 <input type="checkbox"/>	

**Author(s):**

V Gregory Chinchar (chair), Paul Hick, Ikbal Agah Ince, James K Jancovich, Rachel Marschang, Qiwei Qin, Kuttichantran Subramaniam, Thomas B Waltzek, Richard Whittington, Trevor Williams, Qi-Ya Zhang

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**List the ICTV study group(s) that have seen this proposal:**

A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)

Iridoviridae SG

**ICTV Study Group comments (if any) and response of the proposer:**

Drs. Waltzek, Hick, Whittington, Jancovich, Qin, and Chinchar met at the 3<sup>rd</sup> Intl. Symposium on Ranaviruses held May 30 – June 1, 2015 in Gainesville, FL. Consensus was reached on the decision to elevate *Singapore grouper iridovirus* (SGIV) to the status of a species within the genus *Ranavirus* and to propose two subfamilies to differentiate viruses infecting vertebrates from those infecting invertebrates. We also discussed whether to reduce the number of species within the genus *Ranavirus* but will postpone that action for later. In addition, we discussed formation of a 6<sup>th</sup> genus to encompass erythrocytic necrosis viruses from fish, reptiles and amphibians, but will postpone that decision until full genomic sequence data from at least one member of the genus is available.

Date first submitted to ICTV:

27 July 2016

Date of this revision (if different to above):

7 Nov 2016

**ICTV-EC comments and response of the proposer:**

Uc. Label trees to show the proposed subfamilies. State any differences between the subfamilies other than their phylogenetic grouping. [Done]

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be “unassigned” within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	<b>2016.009aD</b>	(assigned by ICTV officers)	
<b>To create new species within:</b>			
Genus:	<b><i>Ranavirus</i></b>	Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “ <b>(new)</b> ” after its proposed name. • If no genus is specified, enter “ <b>unassigned</b> ” in the genus box.	
Subfamily:	<b><i>Alphairidovirinae (new)</i></b>		
Family:	<b><i>Iridoviridae</i></b>		
Order:			
<b>Name of new species:</b>	<b>Representative isolate: (only 1 per species please)</b>	<b>GenBank sequence accession number(s)</b>	
<i>Singapore grouper iridovirus</i>	Singapore grouper iridovirus (SGIV)	AY521625	

**Reasons to justify the creation and assignment of the new species:**

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria.**
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 11

Sequence identity has been one of the criteria used to distinguish species and genera within the family *Iridoviridae*. Generally, genera share less than 50% amino acid identity within a single gene or a group of concatenated genes; species share between 50–90% amino acid identity; members of the same species share 90% or greater sequence identity. Using the type species of the genus *Frog virus 3* (FV3) as a reference, sequence identity between FV3 and SGIV is ~70%. Thus, SGIV is the most divergent member of the genus *Ranavirus* identified to date. A second virus, nearly identical in sequence to SGIV, has been sequenced and this virus, designated grouper iridovirus (GIV), should be considered an isolate or strain of SGIV rather than a distinct species.

MODULE 4: **NEW SUBFAMILY**

creating a new subfamily

A subfamily can only be created within a family.

Code	<b>2016.009bD</b>	(assigned by ICTV officers)
<b>To create a new subfamily within:</b>		
Family:	<i>Iridoviridae</i>	If the family has yet to be created (in Module 5) please write “(new)” after the proposed name. • If there is no Order, write “unassigned” here.
Order:	unassigned	

naming a new subfamily

Code	<b>2016.009cD</b>	(assigned by ICTV officers)
<b>To name the new subfamily: <i>Alphairidovirinae</i></b>		

genera and species assigned to the new subfamily

Code	<b>2016.009dD</b>	(assigned by ICTV officers)
<b>To assign the following genera to the new subfamily:</b>		
You may list several genera here. For each genus, please state whether it is new or existing. <ul style="list-style-type: none"> <li>• If the genus is new, it must be created in Module 3</li> <li>• If the genus already exists, please state whether it is currently unassigned or is to be moved from another family. If the latter, complete Module 7 to ‘MOVE’ it from that family</li> </ul>		
<b>The proposed subfamily <i>Alphairidovirinae</i> includes the following genera: <i>Ranavirus</i> (existing), <i>Megalocytivirus</i> (existing), <i>Lymphocystivirus</i> (existing).</b>		
The new subfamily will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7). <b>Please enter here the TOTAL number of unassigned species that the subfamily will contain (those NOT within any of the genera listed above):</b>		
none		
<b>Reasons to justify the creation of the new subfamily:</b>		
Additional material in support of this proposal may be presented in the Appendix, Module 11		
The subfamily <i>Alphairidovirinae</i> was established to encompass members of the family that infect primarily cold-blooded (ectothermic) vertebrates. Thus, like the poxviruses, host range suggests that the family be divided into two subfamilies: members of the <i>Alphairidovirinae</i> that mainly infect cold-blooded vertebrates, and (described below) members of the <i>Betairidovirinae</i> subfamily that mainly infect invertebrates.		
<b>Origin of the new subfamily name:</b>		
The subfamilies are prefixed with the designations “alpha” (vertebrate hosts) and “beta” (invertebrate hosts) reflecting their dates of “discovery”. Lymphocystis disease, a fish disease characterized by tumor-like growths primarily on the skin, but also internal organs, of affected fish, is caused by lymphocystis disease virus (LCDV), genus <i>Lymphocystivirus</i> . LCDV was first identified in fish more than 100 years ago, whereas invertebrate iridoviruses were first detected in the mid-1950s.		

MODULE 4: **NEW SUBFAMILY**

creating a new subfamily

A subfamily can only be created within a family.

Code	<b>2016.009eD</b>	(assigned by ICTV officers)
<b>To create a new subfamily within:</b>		
Family:	<i>Iridoviridae</i>	If the family has yet to be created (in Module 5) please write “(new)” after the proposed name. • If there is no Order, write “unassigned” here.
Order:	unassigned	

naming a new subfamily

Code	<b>2016.009fD</b>	(assigned by ICTV officers)
<b>To name the new subfamily: <i>Betairidovirinae</i></b>		

genera and species assigned to the new subfamily

Code	<b>2016.009gD</b>	(assigned by ICTV officers)
<b>To assign the following genera to the new subfamily:</b>		
You may list several genera here. For each genus, please state whether it is new or existing. <ul style="list-style-type: none"> <li>• If the genus is new, it must be created in Module 3</li> <li>• If the genus already exists, please state whether it is currently unassigned or is to be moved from another family. If the latter, complete Module 7 to ‘MOVE’ it from that family</li> </ul>		
<b>The proposed subfamily <i>Betairidovirinae</i> includes the following genera: <i>Iridovirus</i> (existing) and <i>Chloriridovirus</i> (existing).</b>		
The new subfamily will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7). <b>Please enter here the TOTAL number of unassigned species that the subfamily will contain (those NOT within any of the genera listed above):</b>		
<b>none</b>		
<b>Reasons to justify the creation of the new subfamily:</b>		
Additional material in support of this proposal may be presented in the Appendix, Module 11		
The subfamily <i>Betairidovirinae</i> was established to encompass members of the family that infect mainly invertebrates. Although there are a few reports describing the infection of captive reptiles and amphibians with insect iridoviruses there are no reports of such infections occurring in nature. Thus, like poxviruses, host range suggests that the family be divided into two subfamilies: members of the <i>Alphairidovirinae</i> that mainly infect cold-blooded vertebrates, and members of the <i>Betairidovirinae</i> subfamily that mainly infect invertebrates.		
<b>Origin of the new subfamily name:</b>		
The subfamilies are prefixed with the designations “alpha” (vertebrate hosts) and “beta” (invertebrate hosts) reflecting their dates of “discovery”. Lymphocystis disease, a fish disease characterized by tumor-like growths primarily on the skin, but also internal organs, of affected fish, is caused by lymphocystis disease virus 1 (LCDV) belonging to species <i>Lymphocystis disease virus 1</i> , genus <i>Lymphocystivirus</i> . LCDV was first identified in fish more than 100 years ago, whereas invertebrate iridoviruses were not detected until the mid-1950s.		

MODULE 7: **MOVE**

Use this module whenever an existing taxon needs to be moved and re-assigned (e.g. when a species is moved from one genus to another).

moving an existing taxon

Code	<b>2016.009hD</b>	(assigned by ICTV officers)
<b>To move the following taxon (or taxa) from their present position:</b>		
<b>The present taxonomic position of these taxon/taxa:</b>		
Genus:	<i>Ranavirus, Megalocytivirus, Lymphocystivirus</i>	Fill in all that apply.
Subfamily:		
Family:	<i>Iridoviridae</i>	
Order:		
Code	<b>2016.009iD</b>	(assigned by ICTV officers)
<b>To re-assign the taxon (or taxa) listed in Part (a) as follows:</b>		
Genus:	<i>Ranavirus, Megalocytivirus, Lymphocystivirus.</i>	Fill in all that apply. <ul style="list-style-type: none"> <li>• If the higher taxon has yet to be created write “(new)” after its proposed name and complete relevant module to create it.</li> <li>If no genus is specified, enter “unassigned” in the genus box.</li> </ul>
Subfamily:	<i>Alphairidovirinae (new)</i>	
Family:	<i>Iridoviridae</i>	
Order:		

**Reasons to justify the re-assignment:**

- If it is proposed to re-assign species to an existing genus, please explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 11

We are proposing a new subfamily to encompass all iridoviruses whose hosts are ectothermic vertebrates (fish, reptiles, and amphibians). A second family (*Betairidovirinae*) has been proposed to contain all iridoviruses that infect invertebrates, e.g., insects, crustaceans, etc. This latter subfamily will contain the *Iridovirus* and *Chloriridovirus* genera.

MOD MODULE 7: **MOVE**

Use this module whenever an existing taxon needs to be moved and re-assigned (e.g. when a species is moved from one genus to another).

moving an existing taxon

Code	<b>2016.009jD</b>	(assigned by ICTV officers)
<b>To move the following taxon (or taxa) from their present position:</b>		
<b>The present taxonomic position of these taxon/taxa:</b>		
Genus:	<i>Iridovirus and Chloriridovirus</i>	Fill in all that apply.
Subfamily:		
Family:	<i>Iridoviridae</i>	
Order:		
Code	<b>2016.009kD</b>	(assigned by ICTV officers)
<b>To re-assign the taxon (or taxa) listed in Part (a) as follows:</b>		
Genus:	<i>Iridovirus and Chloriridovirus</i>	Fill in all that apply. • If the higher taxon has yet to be created write “ <b>(new)</b> ” after its proposed name and complete relevant module to create it. If no genus is specified, enter “ <b>unassigned</b> ” in the genus box.
Subfamily:	<i>Betairidovirinae (new)</i>	
Family:	<i>Iridoviridae</i>	
Order:		

**Reasons to justify the re-assignment:**

- If it is proposed to re-assign species to an existing genus, please explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 11

We are proposing a new subfamily to encompass all iridoviruses whose hosts are ectothermic vertebrates (fish, reptiles, and amphibians). A second family (*Betairidovirinae*) is proposed to contain all iridoviruses that infect invertebrates, e.g., insects, crustaceans, etc. This latter subfamily will contain the *Iridovirus* and *Chloriridovirus* genera.

MODULE 11: **APPENDIX**: supporting material

additional material in support of this proposal

**References:**

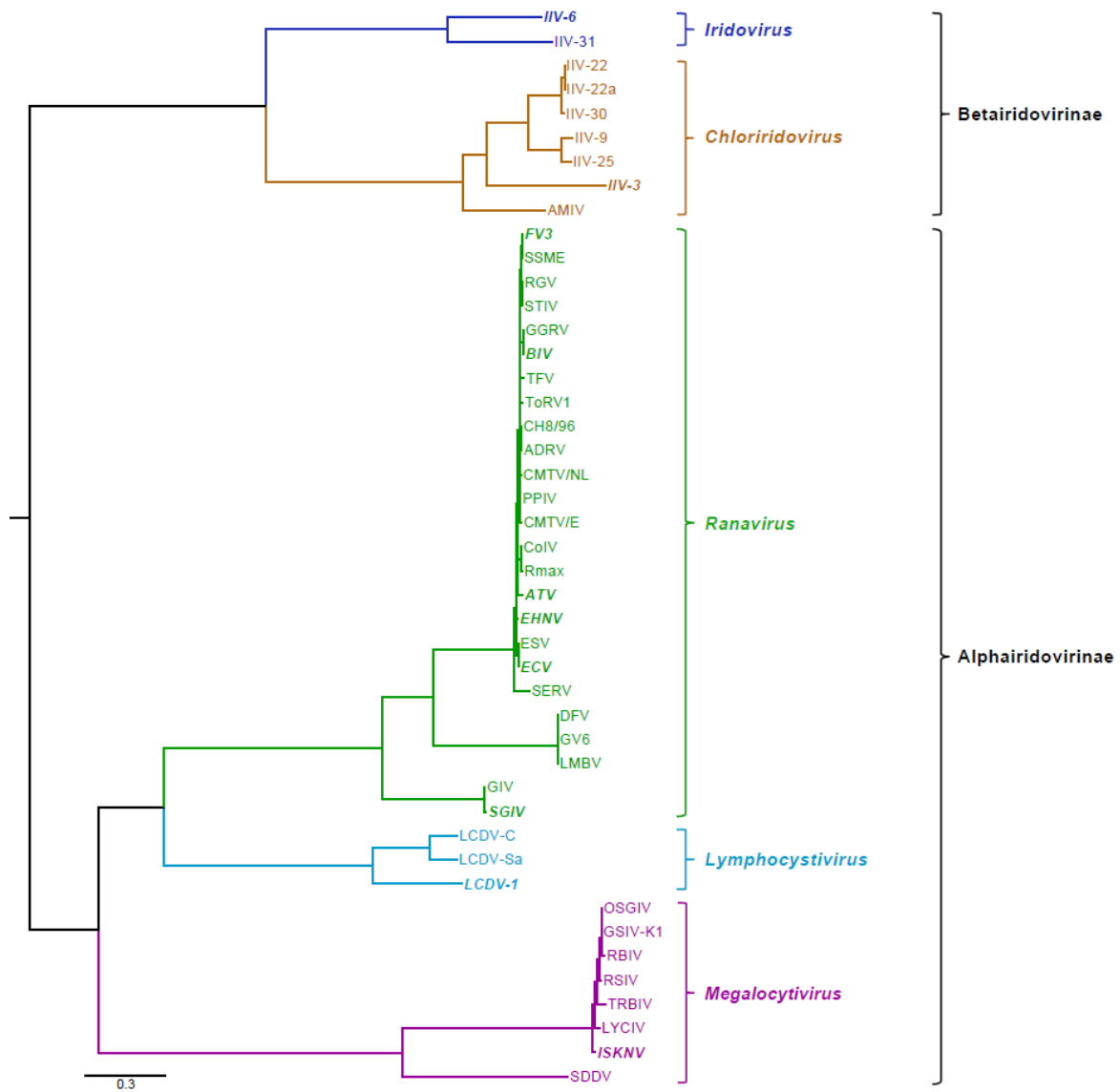
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- Piegu B, Asgari S, Bideshi D, Federici BA, Bigot Y. Evolutionary relationships of iridoviruses and divergence of ascoviruses from invertebrate iridoviruses in the superfamily Megavirales. **Molec. Phylogen. Evol.** 84: 44 – 52 (2015).
- Duffus A, Waltzek TB, Stohr A, Allender M, Gotesman M, Whittington R, Hick P, Hines M, and Marschang RE. Distribution and host range of ranaviruses. In: “**Ranaviruses: Lethal pathogens of ectothermic vertebrates,**” (MJ Gray and VG Chinchar, eds.), Springer OPEN, New York, pp. 9 – 58 (2015).
- Stohr AC, Papp T, Marschang RE. Repeated detection of invertebrate iridoviruses in amphibians. **J. Herpetol. Med. Surg.** 26: 54 – 58 (2016).

**Annex:**

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

Shown below is a phylogenetic tree based on the sequenced genomes of 45 iridoviruses. The phylogram indicates the relationships among the vertebrate (*Alphairidovirinae*) and invertebrate (*Betairidovirinae*) subfamilies within the family *Iridoviridae*. The tree shows a clear division of genera within the *Alphairidovirinae* (*Ranavirus*, green; *Lymphocystivirus*, cyan; and *Megalocytivirus*, purple) and *Betairidovirinae* (*Iridovirus*, blue and *Chloriridovirus*, brown) subfamilies. Moreover, the phylogram supports the assertion that Singapore grouper iridovirus (SGIV) should be considered a new species within the genus *Ranavirus*. Viral species (indicated by their abbreviations) currently recognized by (or proposed for) the ICTV are shown in bold italicized typeface. Species/isolate names and common abbreviations are shown along with

corresponding GenBank accession numbers in Table 1. For tree construction, maximum likelihood analysis was conducted in IQTREE using the concatenated amino acid (AA) sequences of 26 core genes (19,773 AA characters including gaps). The tree was mid-point rooted and the branch lengths are based on the number of inferred substitutions, as indicated by the scale bar. The 26 core genes used in this analysis are those identified previously by Eaton et al. (**Viruses** 2010, 2, 1458 – 1475; doi: 10.3390/v2071458). The tree clearly indicates the division of the family into the two proposed subfamilies as well as relationships among the five recognized genera.



**Fig. 1: Phylogram depicting the relationships between the vertebrate (*Alphairidovirinae*) and invertebrate (*Betairidovirinae*) subfamilies within the family *Iridoviridae*. All branch points (nodes) differentiating the two subfamilies, the five genera, and the separation of SGIV from the other ranaviruses are supported by bootstrap values of 100%.**



**Table 1: Taxonomic Organization of Iridoviruses, Genome Sizes, and GenBank Acc. Nos**

Subfamily	Genus	Species, Strains/Isolates, (Abbreviations)	Size (bp)	GenBank Accession Number
<i>Betairidovirinae</i>	<i>Iridovirus</i>	<b><i>Invertebrate iridescent virus 6</i></b> (IIV-6)	212,482	AF303741
		Invertebrate iridescent virus 31 (IIV-31)	220,222	HF920637
<i>Betairidovirinae</i>	<i>Chloriridovirus</i>	<b><i>Invertebrate iridescent virus 3</i></b> (IIV-3)	191,132	DQ643392
		Invertebrate iridescent virus 9 (IIV-9)	206,791	GQ918152
		Invertebrate iridescent virus 22 (IIV-22)	197,693	HF920633,
		Invertebrate iridescent virus 22A (IIV-22A)	196,455	HF920634
		Invertebrate iridescent virus 25 (IIV-25)	204,815	HF920635
		Invertebrate iridescent virus 30 IIV-30)	198,533	HF920636
		Anopholes minimus iridovirus (AMIV)	163,023	KF938901
<i>Alphairidovirinae</i>	<i>Lymphocystivirus</i>	<b><i>Lymphocystis disease virus 1</i></b> (LCDV-1)	102,653	L63545
		Lymphocystis disease virus China (LCDV-C)	186,250	AY380826
		Lymphocystis disease virus <i>Sparus aurata</i> (LCDV-Sa)	208,501	PRJEB12506
<i>Alphairidovirinae</i>	<i>Ranavirus</i>	<b><i>Frog virus 3</i></b> (FV3)	105,903	AY548484
		Tiger frog virus (TFV)	105,057	AF389451
		<i>Rana grylio</i> virus (RGV)	105,791	JQ654586
		Soft-shelled turtle iridovirus (STIV)	105,890	EU627010
		<b><i>Bohle iridovirus</i></b> (BIV)	103,531	KX185156
		German gecko ranavirus (GGRV)	103,681	KP266742
		<b><i>Ambystoma tigrinum virus</i></b> (ATV)	106,332	AY150217
		<b><i>Epizootic haematopoietic necrosis virus</i></b> (EHNV)	127,011	FJ433873
		European sheatfish virus (ESV)	127,732	JQ724856
		Common midwife toad virus E (CMTV/2008/E )	106,878	JQ231222
		<i>Testudo hermannii</i> ranavirus (CH8/96)	105,811	KP266741
		Tortoise ranavirus 1 (ToRV1)	103,876	KP266743
		Largemouth bass virus (LMBV) **		Unpublished
		<b><i>Singapore grouper iridovirus</i></b> (SGIV)	140,131	AY521625
		Grouper iridovirus (GIV)	139,793	AY666015
		Spotted salamander Maine virus (SSME)	105,070	KJ175144
		Common midwife toad virus NL (CMTV/2013/NL)	107772	KP056312
<i>Andrias davidianus</i> ranavirus (ADRV)	106734	KC865735		
<b><i>European catfish virus</i></b> (ECV)	127549	KT989885		
Short-finned eel ranavirus (SERV)	126965	KX353311		

		<i>Rana maxima</i> virus (Rmax)	115510	KX574343
		Cod iridovirus (CoIV)	114,865	KX574342
		Pike perch iridovirus (PPIV)	108,041	KX574341
		Doctor fish virus (DFV) **		Unpublished
		Guppy virus 6 (GV6)**		Unpublished
<i>Alphairidovirinae</i>	<i>Megalocytivirus</i>	<b><i>Infectious skin and kidney necrosis virus</i> (ISKNV)</b>	111,362	AF371960
		Rock bream iridovirus (RBIV)	112,080	AY532606
		Red seabream iridovirus (RSIV)	112,414	BD143114
		Orange spotted grouper iridovirus (OSGIV)	112,636	AY894343
		Turbot reddish body iridovirus (TRBIV)	110,104	GQ273492
		Lemon yellow croaker iridovirus (LYCIV) *	111,760	AY779031
		Giant seaperch iridovirus K1 (GSIV-K1)	112,565	KT804738
		Scale drop disease virus (SDDV)	124,244	KR139659

\* Conserved genes were identified using NCBI tblastn and ISKNV as the reference genome.

\*\* LMBV, DFV, and GV6 are isolates of ***Santee-Cooper ranavirus***; sequences of the 26 core genes were obtained from unpublished partial genomic sequences.

Table 2: Twenty six core genes within the family *Iridoviridae*

No.	ORF	Putative gene function
1	1R	Putative replication factor and/or DNA binding-packing
2	8R	DNA-dependent RNA polymerase - II largest subunit (Pol II $\alpha$ )
3	9L	Putative NTPase I
4	15R	ATPase-like protein
5	21L	Helicase family
6	22R	D5 family NTPase involved in DNA replication
7	27R	Putative tyrosine kinase/lipopolysaccharide modifying enzyme
8	37R	NIF-NLI interacting factor
9	41R	Hypothetical protein
10	53R	Myristylated membrane protein
11	60R	DNA pol Family B exonuclease
12	62L	DNA-dependent RNA polymerase -II second largest subunit (Pol II $\beta$ )
13	67L	Ribonucleotide reductase small subunit
14	80L	Ribonuclease III
15	84R	Proliferating cell nuclear antigen
16	90R	Major capsid protein
17	95R	Putative XPPG-RAD2-type nuclease
18	19R	Serine-threonine protein kinase
19	57R	Serine-threonine protein kinase
20	2L	Myristylated membrane protein
21	12L	Hypothetical protein
22	81R	Transcription elongation factor TFIS
23	85R	Deoxynucleoside kinase

24	88R	Erv1/Alr family
25	91L	Immediate early protein ICP-46
26	94L	Hypothetical protein- <i>Clostridium tetani</i>

The ORF assignment corresponds to the position of the above genes within the genome of FV-3 (Eaton et al., 2010).

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