

FAMILY IRIDOVIRIDAE

TAXONOMIC STRUCTURE OF THE FAMILY

Family	<i>Iridoviridae</i>
Genus	<i>Iridovirus</i>
Genus	<i>Chloriridovirus</i>
Genus	<i>Ranavirus</i>
Genus	<i>Lymphocystivirus</i>
Genus	<i>Megalocytivirus</i>

VIRION PROPERTIES

MORPHOLOGY

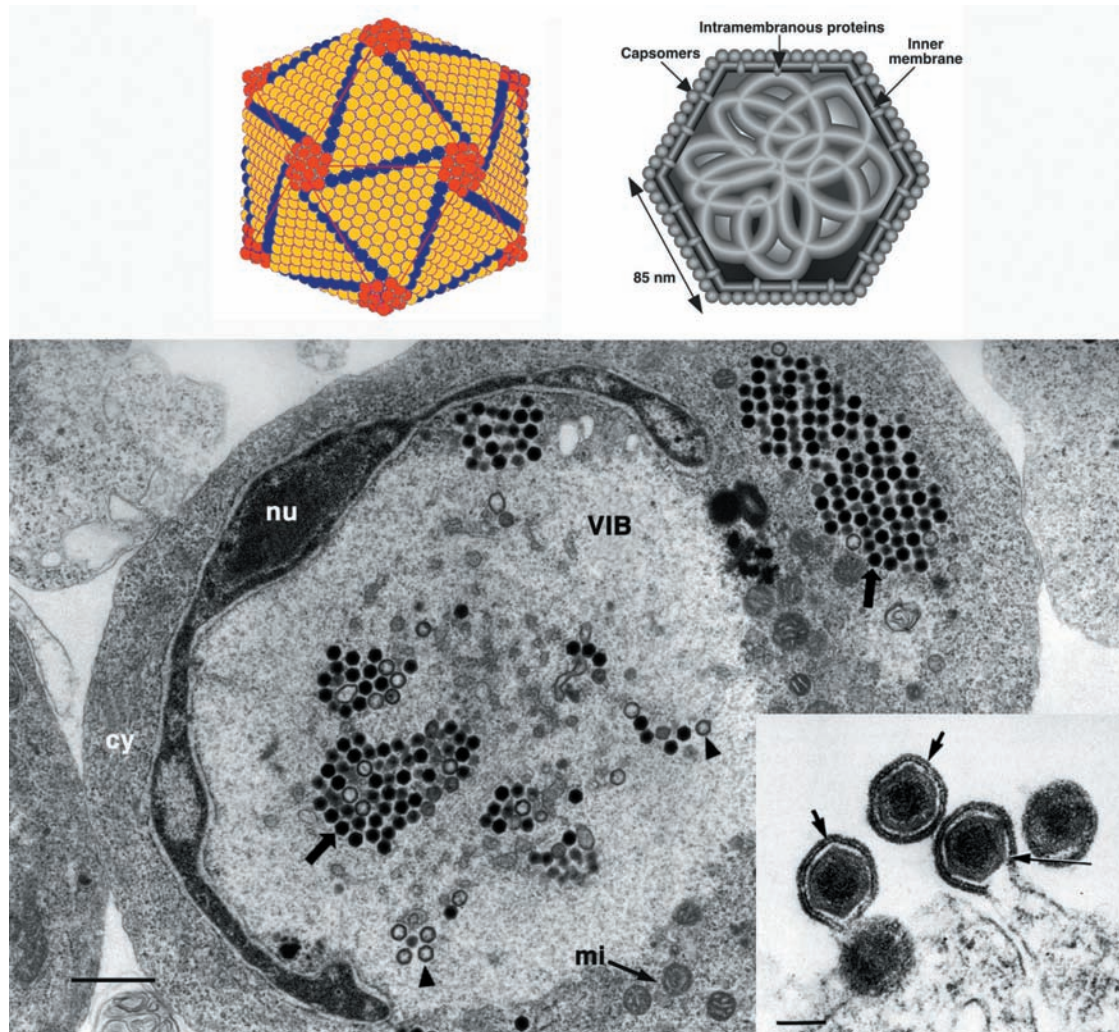


Figure 1: (Top left) Outer shell of Invertebrate iridescent virus 2 (IIV-2) (From Wrigley, *et al.* (1969). *J. Gen. Virol.*, 5, 123. With permission). (Top right) Schematic diagram of a cross-section of an iridovirus particle, showing capsomers, transmembrane proteins within the lipid bilayer, and an internal filamentous nucleoprotein core (From Darcy-Tripier, F. *et al.* (1984). *Virology*, 138, 287. With permission). (Bottom left) Transmission electron micrograph of a fat head minnow cell infected with an isolate of *European catfish virus*. Nucleus (Nu); virus inclusion body (VIB); paracrystalline array of non-enveloped virus particles (arrows); incomplete nucleocapsids (arrowheads); cytoplasm (cy); mitochondrion (mi). The bar represents 1 μm . (From Hyatt *et al.* (2000). *Arch. Virol.* 145, 301, with permission). (insert) Transmission electron micrograph of particles of Frog virus 3 (FV-3), budding from the plasma membrane. Arrows and arrowheads identify the viral envelope (Devauchelle *et al.* (1985). *Curr. Topics Microbiol. Immunol.*, 116, 1, with permission). The bar represents 200 nm.

Virions display icosahedral symmetry and are usually 120-200 nm in diameter, but may be up to 350 nm (e.g. genus *Lymphocystivirus*). The core is an electron-dense entity consisting of a nucleoprotein filament surrounded by a lipid membrane containing transmembrane proteins of unknown function. The capsid is composed of identical capsomers, the number of which depends on virion size. Capsomers are organized to form trisymmetrons and pentasymmetrons in members of the *Iridovirus* and *Chloriridovirus* genera. Capsomer dimensions are approximately 6-7 nm in diameter and 7-13 nm in height. Each capsomer is composed of an internal and external protein trimer. Fibers or short fibrils have been observed trailing from the capsid in viruses from both vertebrate and invertebrate genera. Iridoviruses may acquire an envelope by budding through the host cell membrane. The envelope increases the specific infectivity of virions, but is not required for infectivity as naked particles are also infectious (Fig. 1).

PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The Mr of virions is $1.05\text{--}2.75 \times 10^9$, their sedimentation coefficient ($S_{20,w}$) is 2020-4460S, and their density is 1.26-1.6 g/cm³. Virions are stable in water at 4°C for extended periods. Sensitivity to pH varies, whereas sensitivity to ether and chloroform depends on the assay system employed. All viruses are inactivated within 30 min at >55°C. FV-3, Infectious spleen and kidney necrosis virus (ISKNV), and Invertebrate iridescent virus 6 (IIV-6) are inactivated by UV-irradiation. Some ranaviruses remain infectious after desiccation, e.g., Bohle iridovirus (BIV) survives desiccation at temperatures up to 42°C for up to 6 weeks, whereas others are sensitive to drying.

NUCLEIC ACID

The virion core contains a single linear dsDNA molecule of 140-303 kbp, a value which includes both unique and terminally redundant sequences. Invertebrate iridescent virus 1 (IIV-1) has been reported to have an additional genetic component of 10.8 kbp which exists as a free molecule in the particle core. DNA comprises 12-16% of the particle weight, and the G+C content ranges from ~28 to ~55%. All viruses within the family possess genomes that are circularly permuted and terminally redundant. However, the DNA of vertebrate iridoviruses (members of the genera *Ranavirus*, *Lymphocystivirus* and *Megalocytivirus*) is highly methylated, whereas little to no methylation is found within the genomes of the invertebrate iridoviruses (members of the genera *Iridovirus* and *Chloriridovirus*). The complete genomic sequence is known for Lymphocystis disease virus 1 (LCDV-1), IIV-6, Tiger frog virus (TFV), ISKNV, and Ambystoma tigrinum virus (ATV). Sequence analysis of Frog virus 3 (FV-3) and Epizootic haematopoietic necrosis virus (EHNV) is ongoing. Although naked genomic DNA is not infectious, non-genetic reactivation of viral DNA can be achieved in the presence of viral structural proteins.

PROTEINS

Iridoviruses are structurally complex, and up to 36 polypeptides, ranging from ~5–250 kDa, have been detected by two dimensional PAGE of virus particles. Sequence analysis has identified more than 100 ORFs (Tables 1 and 2, Fig. 2). The major CP (MCP), 48-55 kDa, comprises 40% of the total virion protein, and its complete aa sequence is known for several viruses. The MCP is highly conserved and shares aa sequence identity with the MCPs of African swine fever virus (ASFV, family *Asfarviridae*), several members of the family *Ascoviridae*, and Paramecium bursaria Chlorella virus 1 (PBCV-1, family *Phycodnaviridae*). At least 6 DNA associated polypeptides have been identified in the core of IIV-6, with a major species of 12.5 kDa. A virion-associated protein elicits the shutdown of host macromolecular synthesis, whereas other virion-associated proteins transactivate early viral transcription. A number of virion-associated enzymatic activities have been detected including a protein kinase, nucleotide phosphohydrolase, a ss/dsRNA-specific ribonuclease, pH 5 and pH 7.5 deoxyribonucleases, and a protein phosphatase. In addition to these polypeptides, various other proteins have been identified by BLAST analysis of recently sequenced viral genomes (Table 2; Fig 2).

LIPIDS

Non-enveloped particles contain 5-17% lipid, predominantly as phospholipid. The composition of the internal lipid membrane suggests that this membrane is not derived from host membranes but is produced *de novo*. Viruses released from cells by budding acquire their outer envelope from the plasma membrane.

CARBOHYDRATES

Carbohydrates are not present in purified virions.

GENOME ORGANIZATION AND REPLICATION

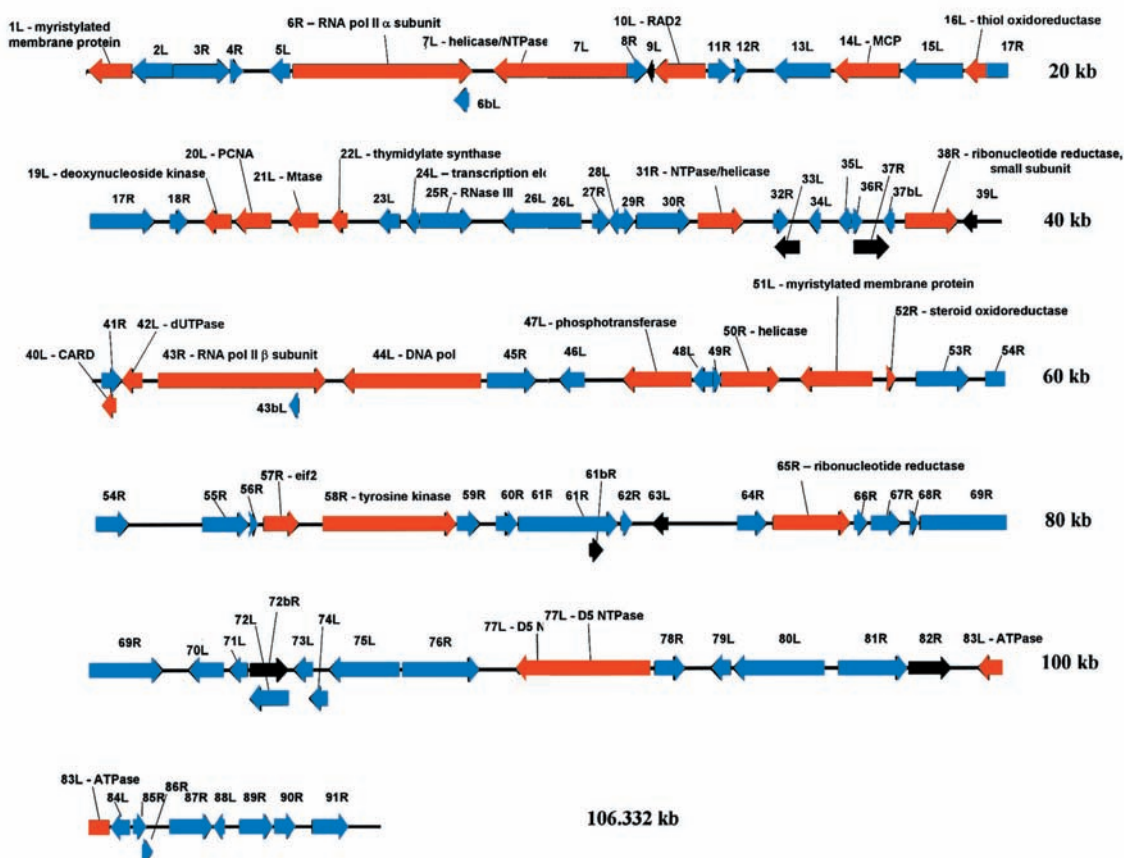


Figure 2: Genomic structure of *Ambystoma tigrinum* virus (ATV). Arrows represent viral ORFs with their size, position, and orientation shown. ORFs of known function are colored in red and their putative proteins identified; ORFs with known homology to Tiger frog virus (TFV) are in blue; and those of unknown function or with no homology to TFV are indicated in black. (Jancovich, and colleagues, unpublished).

The replication strategy of iridoviruses is novel and has been elucidated primarily through the study of FV-3, the type species of the genus *Ranavirus*. Virion entry occurs by either receptor mediated endocytosis (enveloped particles) or by uncoating at the plasma membrane (naked virions). Following uncoating, viral cores enter the nucleus where 1st stage DNA synthesis and the synthesis of immediate early (IE) and delayed early (DE) viral transcripts takes place. In a poorly understood process, one or more virion associated proteins act as transactivators and re-direct host RNA polymerase II to synthesize IE and DE viral mRNAs using the methylated viral genome as template. Gene products encoded by IE and DE viral transcripts include both regulatory and catalytic proteins. One of these gene products, the viral DNA polymerase, catalyzes the 1st stage of viral DNA synthesis. In this process, the parental viral genome serves as the template and progeny DNA that is genome-length, to at most twice genome length, is produced. Newly-synthesized viral DNA may serve as the template for additional rounds of DNA

replication or early transcription, or it may be transported to the cytoplasm where the 2nd stage of viral DNA synthesis occurs. In the cytoplasm, viral DNA is synthesized into large, branched concatamers that serve as the template for DNA packaging.

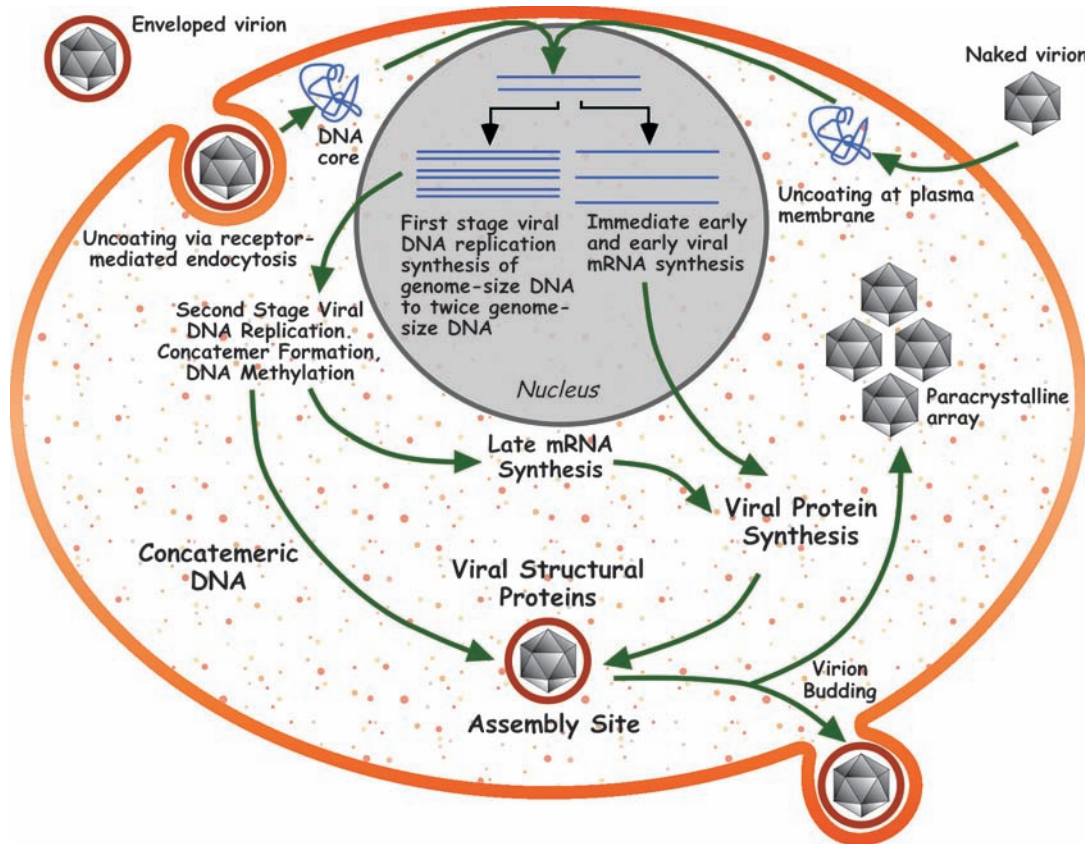


Figure 3: Replication cycle of Frog virus 3 (FV-3)(From Chinchar *et al.*, (2002). *Arch. Virol.*, **147**, 447, with permission).

Table 1. Summary of genomic sequence information for five virus species representing four genera within the family *Iridoviridae*.

Genus Virus species	<i>Iridovirus</i> IIV-6	<i>Ranavirus</i> ATV	<i>Ranavirus</i> TFV	<i>Lymphocysti-</i> <i>virus</i> LCDV-1	<i>Megalocyti-</i> <i>virus</i> ISKNV
Genome size (bp)	212,482	106,332	105,057	102, 653	111,362
G+C%	28.6%	54%	55%	29.1%	54.8%
Putative ORFs	468	102	105	195	124
ORF size (aa)	40 - 2432	40 - 1294	40 - 1294	40 - 1199	40 - 1208
Accession No.	AF303741	AY150217	NC003407	NC001824	NC003494

Viral DNA methylation also likely occurs in the cytoplasm and, although its precise role is uncertain, is thought to protect viral DNA from endonucleolytic attack. Transcription of late (L) viral transcripts occurs in the cytoplasm and full L gene transcription requires prior DNA synthesis. Homologs of the two largest subunits of RNA polymerase II are encoded by all iridoviruses. Whether these function only in the cytoplasm to transcribe L viral transcripts, or whether they also play a role in continued early transcription has not yet been determined. Virion formation takes place in the cytoplasm within

morphologically distinct virus assembly sites. Within assembly sites concatameric viral DNA is packaged into virions via a "headful" mechanism that results in the generation of circularly permuted and terminally redundant genomes similar to those seen with the Enterobacteria phages T4 or P22. The degree of terminal redundancy varies from approximately 5 to 50%. Following assembly, virions accumulate in the cytoplasm within large paracrystalline arrays or acquire an envelope by budding from the plasma membrane. In the case of most vertebrate iridoviruses, the majority of virions remain cell associated (Fig 3).

Table 2. Partial listing of putative gene products encoded by viruses within the genera *Iridovirus*, *Ranavirus*, *Lymphocystivirus* and *Megalocytivirus*.

Category Gene Product	Irido IIV-6	Rana ATV	Rana TFV	Lympho LCDV-1	Megalo ISKNV
Enzymes Associated with Nucleic Acid Replication and Metabolism					
DNA polymerase	+	+	+	+	+
RNA polymerase II, α subunit	+	+	+	+	+
RNA polymerase II, β subunit	+	+	+	+	+
Transcription factor-like protein	+	+	+	+	
RAD-2, DNA repair enzyme		+	+	+	+
Cytosine DNA methyltransferase		+	+	+	+
Type II restriction enzyme <i>Msp</i> I of <i>Moraxella</i> sp.				+	
RNAse III	+	+	+	+	+
Ribonucleotide reductase, large subunit	+	+	+	+	
Ribonucleotide reductase, small subunit	+	+	+	+	+
DUTPase	+	+	+		
Thymidylate synthase	+	+	+		
Thymidine kinase	+			+	
Thymidylate kinase	+	+			
Topoisomerase II-like protein	+				
Helicase	+	+	+		
PCNA protein	+	+		+	
DNA ligase	+				
Additional Enzymatic Activities					
Tyrosine phosphatase	+				
Tyrosine kinase	+	+			
Thiol oxidoreductase		+			
Serine/threonine protein kinase	+			+	+
ATPase	+	+	+	+	+
Matrix metalloproteinase	+				
mRNA capping enzyme					+
Cathepsin B-like protein	+			+	
Putative Immune Evasion Proteins					
TNF receptor-associated factor					+
Growth factor/cytokine receptor family signature	+				
TNFR/NGFR family proteins				+	
PDGF/VEGF-like protein					+
Apoptosis inhibitor (IAP) of <i>Cydia pomonella</i> granulosis virus, Bir repeat profile	+				
IAP-like protein of African swine fever virus	+				

CARD, caspase recruitment domain		+			
3' β hydroxysteroid oxidoreductase		+	+	+	
eIF-2 α homolog		+	+		
Src homology domain, suppressor of cytokine signaling					+
Structural Proteins					
Major CP	+	+	+	+	+
Myristylated membrane protein		+			

The presence of a homolog of the indicated gene is indicated by a plus sign (+).

ANTIGENIC PROPERTIES

The genera are serologically distinct from one another. In the genus *Iridovirus* there exists one main group of serologically interrelated species and others which have little sero-relatedness. Several amphibian isolates (e.g., *Rana esculenta iridovirus*, REIR) and piscine isolates (e.g. EHNV) show serological cross-reactivity with FV-3 (genus *Ranavirus*). Antibodies prepared against virions are often non-neutralizing.

BIOLOGICAL PROPERTIES

Iridoviruses have been isolated only from poikilothermic animals, usually associated with damp or aquatic environments, including marine habitats. Iridovirus species vary widely in their natural host range and in their virulence. Transmission mechanisms are poorly understood for the majority of these viruses. Invertebrate iridoviruses may be transmitted by endoparasitic wasps or parasitic nematodes. Viruses may be transmitted experimentally by injection or bath immersion, and naturally by co-habitation, feeding, or wounding. While many of these viruses cause serious, life-threatening infections, subclinical infections are common.

GENUS *IRIDOVIRUS*

Type Species *Invertebrate iridescent virus 6*

DISTINGUISHING FEATURES

VIRION PROPERTIES

MORPHOLOGY

Particle diameter is 120-130 nm in ultrathin section. IIV-1 and IIV-2 are assumed to contain 1472 capsomers arranged in 20 trimers and 12 pentamers.

PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions have an Mr of approximately 1.28×10^9 , a buoyant density of 1.30-1.33 g/cm³, and a sedimentation coefficient $S_{20,w}$ of 2020-2250S. IIV-6 is sensitive to chloroform, SDS, sodium deoxycholate, ethanol, pH3 and pH11, but is not sensitive to trypsin, lipase, phospholipase A2 or EDTA. The sensitivity of IIV-6 to ether differs depending on whether an *in vivo* or *in vitro* assay is used to determine residual infectivity.

NUCLEIC ACID

Genome sizes vary from 140-210 kbp. This figure includes both unique sequences and a variable amount of terminal redundancy. The unit length genome size of IIV-6 is 212,482 bp. The G+C content is typically 29-32%.

PROTEINS

Two dimensional SDS-PAGE of IIV-6 has revealed the presence of approximately 35 polypeptides ranging from 11 to 300 kDa. Sequence analysis identified 468 putative ORFs of which 234 are non-overlapping. The CP exists in two forms, a 50 kDa

monomeric entity located on the external surface of the capsid and a disulphide-linked homotrimer located in the interior of the capsid.

LIPIDS

The internal lipid layer is believed to be important in the stability of these viruses in aquatic environments. Treatments with chloroform reduces the infectivity of IIV-6 and *Anticarsia gemmatalis* iridescent virus (AGIV) whereas IIV-2 has been reported as insensitive to treatment with ether or chloroform.

CARBOHYDRATES

Carbohydrates are not present in purified virions.

ANTIGENIC PROPERTIES

Genetic studies have indicated the presence of discrete complexes of inter-related viruses within this genus: one large complex containing 10 tentative species, and two smaller complexes. Serological relationships follow a similar pattern.

BIOLOGICAL PROPERTIES

Iridoviruses have been isolated from a wide range of arthropods, particularly insects in aquatic or damp habitats. Patently infected animals and purified viral pellets display violet, blue or turquoise iridescence. Non-apparent, non-lethal infections may be common in certain hosts. No evidence exists for transovarial transmission and where horizontal transmission has been demonstrated, it is usually by cannibalism or predation of infected invertebrate hosts. Following experimental injection, many members of the genus can replicate in a large number of insects. In nature, the host range appears to vary but there is evidence, for some viruses, of natural transmission across insect orders and even phyla. Invertebrate iridescent viruses have a global distribution.

LIST OF SPECIES DEMARCATION CRITERIA IN THE GENUS

The following species-defining characteristics and associated limits are preliminary in nature. The following definitions assume that all material being compared has been grown under near identical conditions and prepared for examination following identical protocols. It is recommended that members of both recognized virus species be included in all characterization studies of novel isolates.

- Amino acid sequence analysis of the MCP: Members of distinct species should exhibit no more than 90% aa sequence identity for the complete protein sequence. PCR primers have been designed for conserved regions of this gene. Although the complete IIV-6 genome has been determined and the sequence of a number of other proteins from different isolates has been ascertained, this information has not been used for species differentiation and quantitative limits of similarity have not been established.
- DNA-DNA dot-blot hybridization: Hybridization values should be less than 50% for members of distinct species. DNA-DNA reassociation in solution has not proven useful for species comparisons of iridoviruses.
- RFLP: Using a panel of not fewer than 4 restriction endonucleases (both rare and frequent cutters) distinct species should show completely distinct restriction endonuclease profiles.
- Serology: Antisera from members of strains within a species should exhibit high levels of cross reactivity. Within and among species, comparison by Western blot analysis using antibodies raised against disrupted virions is the preferred method. Comparisons should be performed simultaneously wherever possible and reference species should be included in each determination.

The major structural protein of IIV-1 shows 66.4% aa sequence identity to that of IIV-6 and approximately 50% or lower aa sequence identity to iridoviruses in other genera. Less than 1% DNA-DNA hybridization for genomic DNA was detected by dot-blot method

between IIV-1 and IIV-6 (stringency: 26% mismatch). Restriction endonuclease profiles (*Hind*II, *Eco*RI, *Sal*I) showed a coefficient of similarity of <66% between IIV-1 and IIV-6. These species did not share common antigens when tested by tube precipitation, infectivity neutralization, reversed single radial immunodiffusion or enzyme-linked immunosorbant assay. Genome and protein size differences are not useful in differentiating these species; genome sizes can be highly variable among strains of a species whereas the size of the MCP is well conserved among species. Little is known about the usefulness of other proteins as species demarcation criteria in the genus.

LIST OF SPECIES IN THE GENUS

Species names are in green italic script; strain names and synonyms are in black roman script; tentative species names are in blue roman script. Sequence accession numbers, and assigned abbreviations () are also listed.

SPECIES IN THE GENUS

Invertebrate iridescent virus 1

Invertebrate iridescent virus 1 [M33542, M62953] (IIV-1)
(*Tipula iridescent virus*) (TIV)

Invertebrate iridescent virus 6

Gryllus iridovirus (GRIV)
Invertebrate iridescent virus 6 [AF003534; M99395] (IIV-6)
(*Chilo iridescent virus*) (CIV)

TENTATIVE SPECIES IN THE GENUS

Anticarsia gemmatalis iridescent virus [AF042343] (AGIV)

Invertebrate iridescent virus 2 [AF042335] (IIV-2)

Sericesthis iridescent virus

Invertebrate iridescent virus 9 [AF025774] (IIV-9)

Invertebrate iridescent virus 10

Invertebrate iridescent virus 18

Opogonia iridescent virus

Wiseana iridescent virus

Witlesia iridescent virus

Invertebrate iridescent virus 16 [AF025775] (IIV-16)

Costelytra zealandica iridescent virus

Invertebrate iridescent virus 21 (IIV-21)

Heliothis armigera iridescent virus

Insect iridescent virus 28

Lethocerus columbinae iridescent virus

Invertebrate iridescent virus 22 [AF042341; M32799] (IIV-22)

Simulium sp. iridescent virus

Invertebrate iridescent virus 23 [AF042342] (IIV-23)

Black beetle iridescent virus

Heteronychus arator iridescent virus

Invertebrate iridescent virus 24 [AF042340] (IIV-24)

Apis iridescent virus

Bee iridescent virus

Invertebrate iridescent virus 29 [AF042339] (IIV-29)

Tenebrio molitor iridescent virus

Invertebrate iridescent virus 30 [AF042336] (IIV-30)

Helicoverpa zea iridescent virus

Invertebrate iridescent virus 31 [AF042337; AJ279821] (IIV-31)

Armadillidium vulgare iridescent virus

Invertebrate iridescent virus 32

Isopod iridescent virus

Porcellio dilatatus iridescent virus

GENUS **CHLORIRIDOVIRUS****Type Species** *Invertebrate iridescent virus 3***DISTINGUISHING FEATURES****VIRION PROPERTIES****MORPHOLOGY**

Particle diameter is approximately 180 nm in ultrathin section. The trimers and pentamers of Invertebrate iridescent virus 3 (IIV-3) are larger than the corresponding structures of the genus *Iridovirus*, with probably 14 capsomers to each edge of the trimer. Particle size has historically been used to define viruses that are members of this genus, but the validity of that characteristic is uncertain.

PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions have a Mr of approximately $2.49\text{-}2.75 \times 10^9$, a buoyant density of approximately 1.354 g/cm^3 in CsCl, and a S_{20w} of 4440-4460S. Infectivity is believed not to be sensitive to ether.

NUCLEIC ACID

The genome size is estimated to be ~135 kbp with a G+C content of 53.9%.

PROTEINS

Protein studies of chloriridoviruses are ongoing. Based on genome size, IIV-3 likely encodes ~100 proteins. Recent sequence analysis has identified a DNA polymerase delta-like protein, and SDS-PAGE detected a 55 kDa protein that is likely the MCP.

LIPIDS

The lipid content of IIV-3 is approximately 4%.

CARBOHYDRATES

None reported.

ANTIGENIC PROPERTIES

IIV-3 is serologically distinct from members of other genera.

BIOLOGICAL PROPERTIES

IIV-3 is the only virus characterized from this genus, although more than 20 host species were reported with patent infections world-wide. Chloriridovirus-like infections have only been reported from Diptera with aquatic larval stages, mainly mosquitoes. There is evidence for transovarial transmission in mosquitoes infected by IIV-3. Horizontal transmission is achieved by cannibalism or predation of infected mosquitoes of other species. Patently infected larvae and purified pellets of virus iridesce usually with a yellow-green color, although orange and red infections are known. IIV-3 appears to have a narrow host range compared to most members of the genus *Iridovirus*.

LIST OF SPECIES DEMARCATION CRITERIA IN THE GENUS

Not applicable.

LIST OF SPECIES IN THE GENUS

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SPECIES IN THE GENUS

Invertebrate iridescent virus 3

Invertebrate iridescent virus 3
Aedes taeniorhynchus iridescent virus
Mosquito iridescent virus

[AJ312708]

(IIV-3)

TENTATIVE SPECIES IN THE GENUS

None reported.

GENUS *RANAVIRUS*

Type Species *Frog virus 3*

DISTINGUISHING FEATURES

VIRION PROPERTIES

MORPHOLOGY

Particle diameter is approximately 150 nm in ultrathin section. Enveloped virions, released by budding, measure 160-200 nm in diameter. The capsid has a skewed symmetry with a T=133 or 147. The internal lipid bilayer contains transmembrane proteins. The nucleoprotein core consists of a long coiled filament 10 nm wide.

PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Buoyant density is 1.28 g/cm³ for enveloped particles and 1.32 g/cm³ for unenveloped particles. Infectivity is rapidly lost at pH 2.0-3.0 and at temperatures above 50°C. Particles are inactivated by treatment with ether, chloroform, sodium deoxychlorate, and phospholipase A.

NUCLEIC ACID

Virions contain a single linear dsDNA molecule of 150-170 kbp. The genome is circularly permuted and approximately 30% terminally redundant. The unit genome size is approximately ~105 kbp with a G+C content of ~54% (Table 1). Cytosines within the dinucleotide sequence CpG are methylated by a virus-encoded cytosine DNA methyltransferase. DNA methylation likely occurs in the cytoplasm and may be important in protecting DNA from viral endonucleases.

PROTEIN

Approximately 30 structural proteins have been detected in FV-3 virions, whereas sequence analysis of TFV, and ATV predicted slightly more than 100 ORFs (Table 1) Proteins do not undergo extensive post-translational processing and no evidence for glycosylation, sulfation or cleavage from precursors has been detected. Phosphoproteins of 10-114 kDa are present in the virion core and a virion-associated protein kinase (44 kDa) has been isolated. Other virion-associated proteins include a nucleotide phosphohydrolase, pH5 and pH7.5 endodeoxyribonucleases, an endoribonuclease, a protein phosphatase, two proteins (VP44 and VP63) within the lipid layer, and one protein (VP58) within the viral envelope. All ranaviruses examined to date appear to encode a viral homolog of eIF-2 α that is thought to play a role in maintaining viral protein synthesis in infected cells. Sequence analysis of key proteins among various species within the genus *Ranavirus* shows high levels (i.e., >70%) of sequence identity.

LIPIDS

Non-enveloped particles contain 9% lipid. The composition of the internal lipid membrane differs from that of the host cell. Viruses released from cells by budding acquire their envelope from the plasma membrane.

CARBOHYDRATES

None reported.

GENOME ORGANIZATION AND REPLICATION

The replication cycle of FV-3 serves as the model for iridoviruses and has been discussed above (Fig. 3). The complete genomes of two ranaviruses (e.g., TFV and ATV) have recently been sequenced and, while possessing homologous proteins, are not co-linear (Table 1, Fig. 2). Sequence analysis of FV-3 and EHNV is ongoing.

ANTIGENIC PROPERTIES

Ranaviruses such as FV-3 are serologically and genetically distinct from members of other genera. However, several piscine, reptilian and amphibian ranavirus isolates show serological and/or genetic relatedness to FV-3

BIOLOGICAL PROPERTIES

Viral transmission occurs by feeding, parenteral injection, or environmental exposure. Ranaviruses grow in a wide variety of cultured fish, amphibian, and mammalian cells, and cause marked cytopathic effect culminating in cell death, likely by apoptosis. In contrast to their marked pathogenicity *in vitro*, their effect in animals depends on the viral species, and on the identity and age of the host animal. For example, Largemouth bass virus (LMBV) shows evidence of wide-spread infection in the wild, but is only rarely linked to serious disease. Likewise, FV-3 infection leads to death in tadpoles, but often causes only non-apparent infections in adults. It is likely that environmental stress leading to immune suppression increases the pathogenicity of a given iridovirus. Ranavirus infections are often not limited to a single species or taxonomic class of animals. For example, EHNV has been reported to infect at least 13 genera of fish. In addition, BIV, a highly virulent pathogen of the burrowing frog *Lymnodynastes ornatus*, can be experimentally transmitted to fish. Therefore, isolation of a ranavirus from a new host species does not necessarily identify a new viral species. In their most severe disease manifestations, ranaviruses such as FV-3, ATV, European catfish virus (ECV) and EHNV are associated with systemic disease and show marked hemorrhagic involvement of internal organs such as the liver, spleen, kidneys, and gut.

LIST OF SPECIES DEMARCATION CRITERIA IN THE GENUS

Ranaviruses cause systemic disease in fish, amphibians, and reptiles. Members of the six viral species are differentiated from one another by multiple criteria: RFLP profiles, virus protein profiles, DNA sequence analysis, and host specificity. PCR primers have been designed to amplify 3' and 5' regions within the MCP gene for identification purposes. Definitive quantitative criteria based on the above features have not yet been established to delineate different viral species. Generally, if a given isolate shows a distinct RFLP profile, possesses a distinctive host range, and is markedly different from other viruses at the aa sequence level, it is considered a distinct viral species. For example, ranavirus DNA digested with *KpnI* can be ordered into several groups based on RFLP profiles. Strains within the same species shared >70% of their bands in common and showed >95% aa sequence identity within the MCP or other key genes (e.g., ATPase, eIF-2 α homolog).

LIST OF SPECIES IN THE GENUS

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SPECIES IN THE GENUS

<i>Ambystoma tigrinum virus</i>		
Ambystoma tigrinum virus	[AY150217]	(ATV)
Regina ranavirus		
<i>Bohle iridovirus</i>		
Bohle iridovirus	[AF157650, AF157651]	(BIV)
<i>Epizootic haematopoietic necrosis virus</i>		

Epizootic haematopoietic necrosis virus	[U82552, U82631, AJ007358, AJ130963, AJ130964, AJ130965]	(EHNV)
<i>European catfish virus</i>		
European catfish virus	[AF 157678, AF127911]	(ECV)
European sheatfish virus		(ESV)
<i>Frog virus 3</i>		
Box turtle virus 3		
Bufo bufo United Kingdom virus		
Bufo marinus Venezuelan iridovirus 1		
Frog virus 3	[U36913, U15575]	(FV-3)
Lucké triturus virus 1		
Rana temporaria United Kingdom virus		
Redwood Park virus		
Stickleback virus		
Tadpole edema virus		
Tadpole virus 2		
Tiger frog virus	[NC_003407]	(TFV)
Tortoise virus 5		
<i>Santee-Cooper ranavirus</i>		
Doctor fish virus		(DFV)
Guppy virus 6		(GV6)
Santee-Cooper ranavirus (Largemouth bass virus)	[AF080250]	(SCRV) (LMBV)
TENTATIVE SPECIES IN THE GENUS		
<i>Rana esculenta iridovirus</i>		(REIR)
<i>Singapore grouper iridovirus</i>		(SGIV)
<i>Testudo iridovirus</i>		(ThIV)

GENUS *LYMPHOCYSTIVIRUS*

Type Species *Lymphocystis disease virus 1*

DISTINGUISHING FEATURES

VIRION PROPERTIES

MORPHOLOGY

Particle size varies from 198-227 nm for Lymphocystis disease virus 1 (LCDV-1) and 200 nm for LCDV-2. The capsid may show a fringe of fibril-like external protrusions ~ 2.5 nm in length and a double-layered outer envelope.

PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions are heat labile. Infectivity is sensitive to treatment with ether or glycerol.

NUCLEIC ACID

By restriction endonuclease analysis, the genome length is 102.6 kbp for LCDV-1 and approximately 98 kbp in LCDV-2. Contour length measurements by electron microscopy indicate the DNA molecule to be 146 kbp; the degree of terminal redundancy is approximately 50% but varies considerably among virions. The G+C content is 29.1%. Like FV-3, the genome is highly methylated. The presence of 5-methylcytosine occurs at 74% of CpG, 1% of CpC and 2-5% of CpA giving an overall level of methylation of 22%. The complete DNA sequence is known for LCDV-1.

PROTEIN

SDS-PAGE revealed the presence of 33 polypeptides, ranging from 4 to 220 kDa, in LCDV-1 virions isolated directly from flounder tumors. Analysis of LCDV-2 virions gives a discernibly different pattern of polypeptides supporting the idea that they are distinct species. The MCP is 51.4 kDa comprising 459 aa. Enzymatic activities associated with purified virions include a viral encoded adenosine triphosphate hydrolase, a protein kinase and a thymidine kinase. Genome sequence analysis indicated the presence of 38 putative proteins with significant aa sequence homologies to proteins of known function.

LIPID

Non-enveloped particles contain up to 17.1% lipid which is readily digested by treatment with phospholipase A₂, suggesting high levels of phospholipid as seen in other members of the family.

CARBOHYDRATES

None reported.

GENOME ORGANIZATION AND REPLICATION

The LCDV-1 genome contains 195 potential ORFs of which 110 are largely non-overlapping and 38 of which show significant homology to proteins of known function. These 38 ORFs represent 43% of the coding capacity of the genome. The presence of a DNA methyltransferase and a methyl-sensitive restriction endonuclease with specificity for a CCGG target site may be indicative of a restriction-modification system capable of degrading host genomic DNA while protecting viral DNA by specific methylation. LCDV-1 DNA contains numerous short direct, inverted and palindromic repetitive sequence elements. Lack of a suitable cell line has hindered studies of LCDV replication. Virus assembly occurs in and around virogenic stroma in the cytoplasm. Crescent shaped capsid precursors develop into fully formed capsids followed by condensation of the core structures.

ANTIGENIC PROPERTIES

Not known.

BIOLOGICAL PROPERTIES

LCDV-1 infects flounder and plaice while LCDV-2 infects dab. Infection results in benign, wart-like lesions comprising grossly hypertrophied cells occurring mostly in the skin and fins. The disease has been observed in over 100 teleost species although virus species other than LCDV-1 or LCDV-2 may cause a similar clinical disease. The duration of infected cell growth and viral proliferation is highly variable (5 days to 9 months) and is likely temperature dependent. Virions are released following degeneration of the lesions. Transmission is achieved by contact; external sites, including the gills are the principal portals of entry. High host population densities and external trauma are believed to enhance transmission. Implantation and injection are also effective routes of transmission. The incidence of disease may be higher in the presence of certain fish ectoparasites. LCDV is generally not considered of major economic importance. However, although infectious are usually benign and self-limiting, there may be commercial concerns among food and ornamental fish because of market rejection due to the warty appearance of infected animals. Moreover, mortalities may occur under culture conditions, especially when infections involve the gills or when there is debilitation or bacterial infection. Both LCDV-1 and LCDV-2 are difficult to culture *in vitro* although limited growth has been reported in several fish cell lines.

LIST OF SPECIES DEMARCATION CRITERIA IN THE GENUS

Definitive criteria have not yet been established to delineate the viral species. LCDV-1 infects flounder and plaice, while LCDV-2 infects dab. The two species are distinguished from one another by host specificity, histopathology, and molecular criteria: viral protein profiles, DNA sequence analysis, and PCR. PCR primers targeted to regions within the MCP and ORF167L can be used to distinguish between species. While the designation of LCDV-1 as a viral species is well supported in the literature, data supporting the designation of LCDV-2 is still preliminary.

LIST OF SPECIES IN THE GENUS

Species names are in green italic script; strain names and synonyms are in black roman script; tentative species names are in blue roman script. Sequence accession numbers, and assigned abbreviations () are also listed.

SPECIES IN THE GENUS

Lymphocystis disease virus 1

Lymphocystis disease virus 1	[L63545]	(LCDV-1)
(Flounder lymphocystis disease virus)		(FLDV)
Flounder virus		

TENTATIVE SPECIES IN THE GENUS

Lymphocystis disease virus 2	(LCDV-2)
Dab lymphocystis disease virus	

GENUS *MEGALOCYTVIRUS*

Type Species *Infectious spleen and kidney necrosis virus*

DISTINGUISHING FEATURES

VIRION PROPERTIES

MORPHOLOGY

Virions possess icosahedral symmetry and are ~ 140–200 nm in diameter.

PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions are sensitive to heat (56°C for 30 min), sodium hypochlorite, UV irradiation, chloroform and ether, and are variably inactivated by exposure to pH3 and pH11.

NUCLEIC ACID

The complete genomes of ISKNV and Red Sea bream iridovirus (RSIV) have been sequenced. ISKNV virions contain a single, linear dsDNA molecule of 111,362 bp with a G+C content of 54.8%. As with other members of the family, genomic DNA is circularly permuted, terminally redundant, and highly methylated.

PROTEINS

Sequence analysis of the ISKNV genome has identified 124 putative ORFs ranging in size from 40–1208 aa. Thirty five putative proteins with significant aa sequence homologies to proteins of known function in other species have been identified. ATPase and MCP genes (239 and 454 aa, respectively) have also been sequenced from Sea bass iridovirus (SBIV), Grouper sleepy disease iridovirus (GSDIV), Dwarf gourami iridovirus (DGIV), and African lampeye iridovirus (ALIV).

LIPIDS

None reported.

CARBOHYDRATES

None reported.

GENOME ORGANIZATION AND REPLICATION

The complete genome of ISKNV has been sequenced and appears similar in size and gene content to those of other iridoviruses.

ANTIGENIC PROPERTIES

No serotypes are reported and all megalocytiviruses analyzed to date appear to be members of the same viral species. Polyclonal anti-RSIV serum shows cross-reactivity with ESV- and EHNV-infected cells, whereas monoclonal anti-RSIV antibodies react only with RSIV-infected cells. Megalocytiviruses show high levels (i.e. >93%) of aa sequence identity among the proteins characterized to date.

BIOLOGICAL PROPERTIES

Iridoviruses infecting red sea bream, mandarin fish and over 20 other species of marine and tropical fish have been known since the late 1980's. Isolates from red sea bream (RSIV) and the mandarin fish (ISKNV) have been studied extensively. Viral infection is characterized by the formation of inclusion body-bearing cells (IBC). IBCs may be derived from virus-infected macrophages and enlarge by the growth of a unique inclusion body that may be sharply delineated from the host cell cytoplasm by a limiting membrane. When a limiting membrane is seen, the inclusions contain the viral assembly site and mitochondria. IBCs frequently appear in the spleen, hematopoietic tissue, gills, and digestive tract. Necrotized splenocytes are also observed. Transmission has been demonstrated by feeding, parenteral injection, and by environmental exposure. Megalocytiviruses naturally infect and cause significant mortality in freshwater and marine fish in aquaculture facilities in China, Japan, and SE Asia. A partial list of susceptible fish species includes mandarin fish (*Siniperca chuatsi*), red sea bream (*Pagrus major*), grouper (*Epinephelus* spp), yellowtail (*Seriola quinqueradiata*), striped beakperch (*Oplegnathus fasciatus*), red drum (*Sciaenops ocellata*), and African lampeye (*Aplocheilichthys normani*). The virus grows in several cultured piscine cell lines and causes a characteristic enlargement of infected cells. Outbreaks of disease caused by ISKNV only occur in fish cultured at temperatures >20°C. A vaccine targeted to RSIV has been developed.

LIST OF SPECIES DEMARCATION CRITERIA IN THE GENUS

Megalocytiviruses are distinguished from ranaviruses and lymphocystiviruses by their cytopathological presentation (i.e., inclusion body-bearing cells) and sequence analysis of key viral genes, e.g., ATPase and MCP, for which PCR primers have been developed. Megalocytiviruses show >94% sequence identity within these genes, whereas sequence identity with ranaviruses and lymphocystiviruses is <50%. Based on sequence analysis and serological studies, all megalocytiviruses isolated to date appear to be strains of the same viral species.

LIST OF SPECIES IN THE GENUS

Species names are in green italic script; strain names and synonyms are in black roman script; tentative species names are in blue roman script. Sequence accession numbers, and assigned abbreviations () are also listed.

SPECIES IN THE GENUS***Infectious spleen and kidney necrosis virus***

Infectious spleen and kidney necrosis virus	[NC_003494]	(ISKNV)
Red Sea bream iridovirus	[AB006954,AB007366,AB007367,AB018418]	(RSIV)

Sea bass iridovirus	[AB043977]	(SBIV)
African lampeye iridovirus	[AB043979]	(ALIV)
Grouper sleepy disease iridovirus	[AB043978]	(GSDIV)
Dwarf gourami iridovirus		(DGIV)
Taiwan grouper iridovirus	[AAL68652]	(TGIV)

TENTATIVE SPECIES IN THE GENUS

None reported

PHYLOGENETIC RELATIONSHIPS WITHIN THE GENUS

The megalocytiviruses sequenced to date show >95% nt identity and >98% aa sequence identity within the ATPase gene, and >94% nt and >97% aa sequence identity within the MCP gene. In contrast they are distantly related to lymphocystiviruses, ranaviruses, and iridoviruses. For example, the ATPase gene of SBIV shows 54% identity and 72% similarity to that of LCDV-1; 45% identity and 62% similarity to FV-3; and 38% identity and 62% similarity to IIV-6.

LIST OF UNASSIGNED SPECIES IN THE FAMILY

The presence of large, non-enveloped virus particles in both assembly sites and paracrystalline arrays within the cytoplasm of infected cells is characteristic of iridovirus infections. Because of these distinguishing morphological features, several viruses infecting ectothermic animals have been tentatively identified as iridoviruses without further molecular or serological characterization. Furthermore, because many of these viruses have not yet been grown in culture little is known about their mode of replication and molecular organization.

Unassigned viruses within the family include:

White sturgeon iridovirus	(WSIV)
Erythrocytic necrosis virus	(ENV)

PHYLOGENETIC RELATIONSHIPS WITHIN THE FAMILY

Amino-acid sequence analysis of the MCP, DNA polymerase, ATPase, and other genes supports the existence of four genera within the family: *Iridovirus*, *Ranavirus*, *Megalocytivirus*, and *Lymphocystivirus* (Fig. 4). *Chloriridovirus* constitutes a fifth genus within the family, although little sequence information is currently available to support that assertion. Among members of the *Iridoviridae*, MCP and ATPase protein sequence identities are generally >40%.

SIMILARITY WITH OTHER TAXA

The iridovirus MCP and viral DNA polymerase genes share aa sequence similarities to *African swine fever virus* (*Asfarviridae*), species within the family *Ascoviridae*, and *Paramecium bursaria Chlorella virus 1* (*Phycodnaviridae*) (Fig. 4). Although not shown here, more distant relatedness to herpesviruses, adenoviruses, poxviruses, and baculoviruses has been noted.

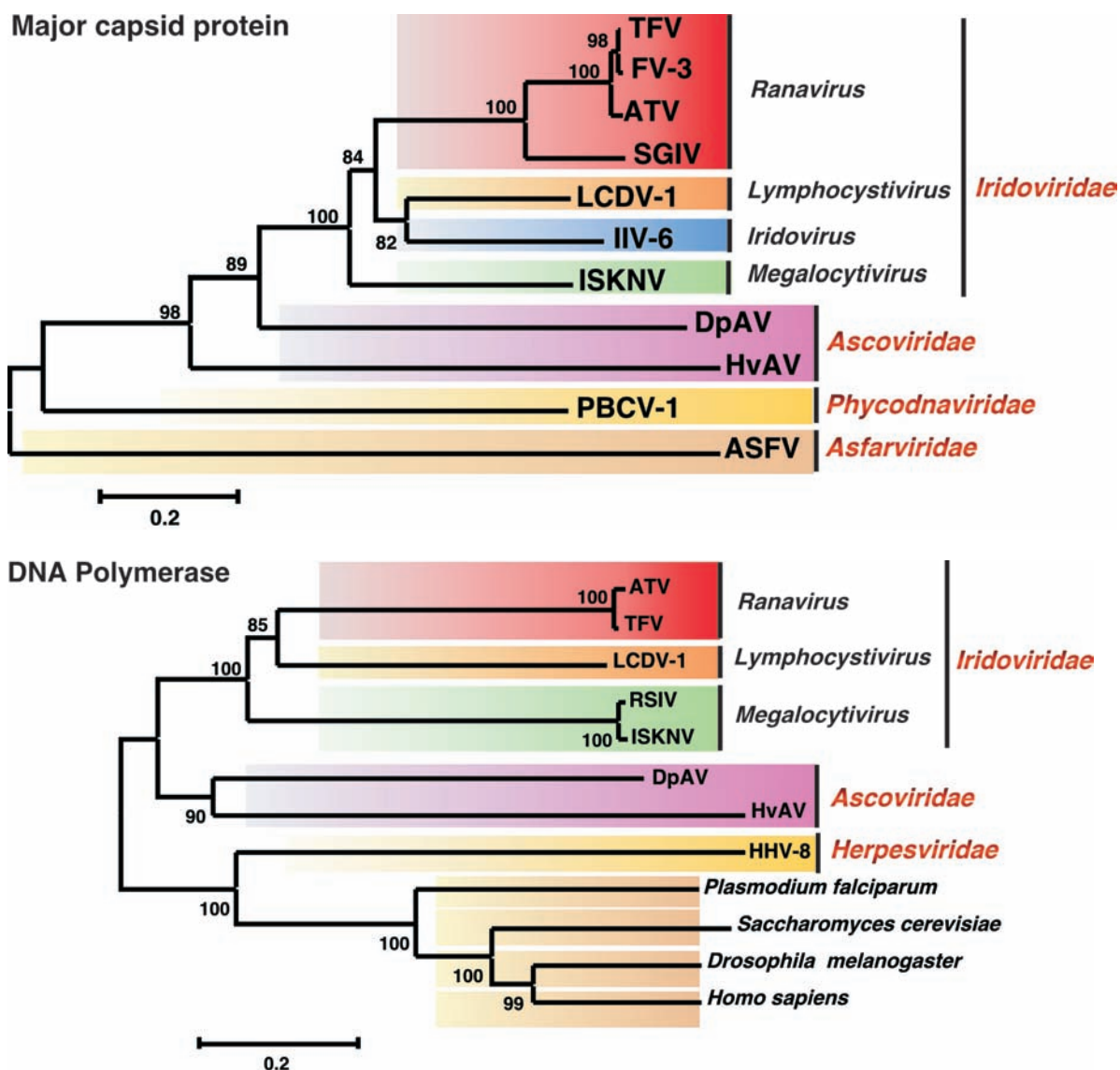


Figure 4: Phylogenetic trees depicting the relationship of selected iridoviruses to other species within the family *Iridoviridae*, viruses outside the family, and host proteins. Top panel: Comparison of the major CPs of four members of the genus *Ranavirus* (TFV, FV3, ATV, and SGIV) to LCDV (genus *Lymphocystivirus*), IIV-6 (genus *Iridovirus*), ISKNV (genus *Megalocytivirus*), two members of the family *Ascoviridae* (*Diadromus pulchellus* ascovirus, DpAV and *Heliothis virescens* ascovirus, HvAV), *Paramecium bursaria* *Chlorella* virus 1 (PBCV-1, *Phycodnaviridae*), and African swine fever virus (ASFV, *Asfarviridae*). Right panel: Comparison of the DNA polymerase gene of ATV and other iridoviruses to the DNA polymerase genes of two ascoviruses (HvAV and DpAV), Human herpesvirus 8 (HHV-8), *Plasmodium falciparum*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Homo sapiens*. (Jancovich *et al.*, (2003), *Virology* 316, 90. With permission).

DERIVATION OF NAMES

Chloro: from Greek *chloros*, meaning "green".

Cysti: from Greek *kystis* meaning "bladder/sac".

Irido: from Greek *iris*, *iridos*, goddess whose sign was the rainbow, hence iridescent "shining like a rainbow" from the appearance of patently infected invertebrates and centrifuged pellets of virions.

Lympho: from Latin *lymphā*, meaning "water".

Megalocyti: from the Greek, meaning "enlarged cell"

Rana: from Latin *rana*, meaning "frog".

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