Ranaviruses of Fish, Amphibians and Reptiles: Diversity and the Requirement for Revised Taxonomy

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ABSTRACT

The genus Ranavirus from the family Iridoviridae includes a limited number of ICTV (International committee on Taxonomy of Viruses) recognised viruses and a larger number of tentative viral assignments. Within the scientific literature an even larger number of ranaviruses, as yet not recognised by the ICTV, have been reported. In this paper we review the major viruses identified within finfish of Australia and discuss the relevance of this in terms of new and emerging viruses, natural viral assemblages and the importance of taxonomy of emerging ranaviruses. We suggest future reviews of the taxonomy of ranaviruses should include universal and polythetic class criteria covering a range of viral and host biotic characteristics. The suggested approach to taxonomy questions the current trend to classify viruses solely by genomic analyses. A suggested approach to classify ranaviruses is outlined whereby iridoviruses from poikilothermic vertebrates can be categorised into genera, species and genotypes.

INTRODUCTION

Over the past 10 to 15 years many new viruses have been identified within Australian poikilothermic vertebrates (Table 1). The identification of these viruses raises many topical questions. For example, where did the viruses come from, what were the circumstances whereby they appeared, are they expanding in their geographic range, are they associated with free-ranging population declines (i.e. amphibian population declines) and are they a threat to commercial aquaculture and trade activities? These questions are complex and will require significant research effort to generate data that may offer some insights into this area of host-virus ecology. To facilitate such research, a prerequisite will be the capacity to accurately identify, characterise and differentiate the pertinent viruses.

To assist in exploring the above questions in the context of the Australian environment it is important to explore the diversity of viruses isolated from Australian poikliotherms. In this paper we shall discuss (A) ranaviruses isolated from Australian poikliotherms (fish and

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reptiles) and discuss these in (B) the context of overseas ranaviruses and the importance of using basic biological data for identifying and differentiating ranaviruses whereby they can be classified into discrete families, sub-families, genera, species and genotypes.

NEW AND EMERGING VIRUSES OF AUSTRALIAN LOWER VERTEBRATES

Within the context of this paper, new viruses are defined as those that have not previously been described; emerging viruses are those that are currently expanding their geographical range (Daszak *et al*., 2000).

Table 1. Viruses identified from Australian fish, amphibians and reptiles.

Host	Virus	Origin	*Associated Disease**	*Endemic/ Exotic*	*Reference*
FISH					
Red-fin perch (Perca fluviatilis)	Epizootic haematopoietic necrosis virus, EHNV (ranavirus)	Victoria, 1985	Y	Endemic	Langdon *et al.* (1986)
Rainbow trout *(Oncorhynchus mykiss)*			Y		
***Barramundi* (Lates calcarifer)**	Lymphocystis virus	Queensland, 1989	Y	Endemic	Pearce *et al.* (1990)
Barramundi (Lates calcarifer)	Barramundi nodavirus	Queensland, 1987	Y	Endemic	Munday *et al.* (1992)
Atlantic salmon (Salmo salar)	Tasmanian aquareovirus	Tasmania, 1990	N	Endemic	Hyatt (unpub.)
Atlantic salmon (Salmo salar)	Tasmanian aquabirnavirus	Tasmania, 1997	N	Endemic	Crane *et al.* (2000)
Flounder (Unknown species)	Herpesvirus	Tasmania, 1996	Y	Endemic (?)	Hyatt (unpub.)
Pilchards (Sardinops sagax)	Herpesvirus	Australian coastline, 1995, 1998	Y	Exotic (?)	Hyatt *et al.* (1997) & Whittington *et al*. (1997)
Pilchards (Sardinops sagax)	Orthomyxo-like virus	South Australia, 1998	N	Unknown	Hyatt (unpub.)
Imported dwarf gourami	'Ranavirus'	Tasmania, 1992	Y	Exotic	Hyatt (unpub.)
Unknown imported ornamental fish	Birnavirus	Victoria, 1987	Y	Exotic	Hyatt (unpub.)
AMPHIBIANS					
Ornate burrowing frog *Limnodynastes ornatus*	Bohle iridovirus, BIV (ranavirus)	Australia	N	Endemic	Spear *et al*., 1991; Hengstberger *et al.* (1993).
REPTILES					
Python *(Aspidites mmelanocephalus)*	Erthrocytic virus	Victoria 1993	Y	Endemic	(Hyatt unpub.)
Green tree python *(Chondropython viridis)*	Wamena virus (ranavirus)	Irian Jaya (illegal import) Queensland*	Y	Exotic	Hyatt *et al*. (2002)

*Disease, defined as animals displaying either abnormal clinical signs, morbidity and/or mortalities.; (?), origin of virus not proved.

Table (1) lists the viruses, which we have identified within a variety of Australian fish, amphibians and reptiles. Most of these viruses are assumed to be endemic as they are yet to be identified outside of Australia. Of these viruses, some are associated with disease whilst others are not (benign). Hyatt *et al*. (2004) discusses the possible significance of endemic, benign viruses in terms of host viral assemblages. From an evolutionary viewpoint it is generally accepted that such viruses have evolved with their hosts and form part of their natural ecology (Hurst, 2000). Based on this hypothesis it is not surprising that we continue to identify 'new' viruses such as Bohle iridovirus (BIV), Australian aquareovirus and Australian aquabirnavirus (Table 1). We should, however, be cautious about the usage of the term 'benign' because such viruses are most likely associated with functions essential to the long-term 'health' of the host ecology (e.g. population dynamics). It should be noted that if selection pressures are present whereby a 'benign' virus can spill-over from their natural to new hosts then the virus can become of overt significance (Daszak *et al*., 2000). Such an example may be EHNV. Experimental trials with EHNV and redfin perch have demonstrated that these fish are exquisitely sensitive to the virus (Langdon et al., 1986; Reddacliff and Whittington, 1996) and are therefore unlikely to be the natural host. Consequently EHNV is most likely a member of the natural viral assemblage of an as yet unidentified animal within the Australian environment. The spill-over of such a virus would occur upon its exposure to naïve hosts (e.g. redfin perch) in conditions of optimum host susceptibility, viral replication and propagation (e.g. status of host immune system, age, population density and temperatures).

Within Table 1 there are also a number of viruses that are associated with clinical disease in free-ranging and/or farmed animals. Of these two viruses, EHNV and Barramundi Nodavirus (*Lates calcarifer* encephalitis virus) appear to be increasing their geographical range and can be referred to as emerging viruses (e.g. Whittington *et al*., 1996; and unpublished data).

Ranaviruses are emerging pathogens of poikliothermic vertebrates

This paper will restrict further discussion to the emerging ranaviruses which encompass a broad collection of viruses from Australia and elsewhere in the world and which collectively have the potential to cause significant mortalities within a broad range of fish, reptiles and amphibians (e.g. Langdon *et al*., 1986; Langdon *et al*., 1988; Moody and Owens, 1994; Jancovich *et al*., 1998; Hyatt *et al*., 1997; Dury *et al*., 1995). Of these viruses, the Office of the International des Epizooties (2002) (OIE) recognises EHN as a notifiable list B disease i.e. a 'transmissible disease that is considered to be of socio-economic and/or public health importance within countries and that is significant in the international trade of animals and animal products'. The identification of other 'iridoviruses' (e.g. WSIV and RSIV) from diseased farmed animals may lead to the similar listing of the associated diseases by the OIE. Infectious diseases of wildlife are also identified by the OIE (Rev. sci. tech. Off. int. Epiz., 2002, 21 (2), 217) as an emerging animal health issue of world-wide importance; as such, ranaviruses associated with disease of free-ranging poikliotheric vertebrates will most-likely be formally recognised by the organisation within the near future. With the increasing recognition of the importance of ranaviruses within wildlife and commercial species, it is obvious that the future identification of 'ranaviruses' will have to be more precise.

Examination of Table 2 shows the number of ranaviruses reported outside Australia. The list is not exhaustive but indicates the large number of viruses and confusion (refer below) that is present in the identification of putative ranaviruses. 'Ranaviruses' have been identified from most continents and the United Kingdom and extend from temperate to tropical waters. Animals include fish (freshwater and marine), reptiles (snakes and turtles) and amphibians (frogs, toads and salamanders). In most descriptions the identifications are associated with disease and death but to date no long-term animal population declines have been documented; the only reported infectious agent that is consistently associated with amphibian population declines is Batrachochytrium dendrobatitis, which causes the fatal epidermal disease chytridiomycosis (Berger *et al*., 1998).

Table 2. Examples of some putative ranaviruses identified in a range of fish, amphibians and reptiles*

HOST	VIRUS#	ORIGIN	REFERENCE
Atlantic cod Gadus morhua		Europe (Denmark)	Jensen *et al*., 1979; Wolf, 1988.
Common carp *Cyprinus carpio*	CCIV	Russia	Popkova & Shchelkunov, 1978; Wolf, 1988.
Catfish *Ictalurus melas*	ECV	Europe (France)	Pozet *et al*., 1992.
Sheatfish *Silurus glanis*	ESV	Europe (Germany)	Ahne *et al*., 1989.
Goldfish *Carassius auratus*	GFV-1	North America (U.S.A.)	Murphy *et al*., 1995; Berry *et al*., 1983.
Goldfish *Carassius auratus*	GFV-2	North America (U.S.A.)	Murphy *et al*., 1995; Berry *et al*., 1983.
Dwarf gourami *Colisa lalia*		Australia# (fish imported from Singapore)	Anderson *et al*., 1993.
Gourami Trichogaster trichopterus		North America (U.S.A.)	Fraser *et al*., 1993.
***Chromide cichlid* Etroplus maculatus**		North America (Canada)# (fish imported from Singapore)	Armstrong *et al*., 1989.
White sturgeon *Acipenser transmontanus*	WSIV	North America (U.S.A.)	Hedrick *et al*., 1992; LaPatra *et al*., 1994.
Red sea bream *Pagrus major*	RSIV	Japan	Inouye *et al*., 1992.
Crimson sea bream *Evynnis japonica*		South-East Asia#	Miyata *et al*., 1997.
Sea bass *Lateolabrax sp.*		Japan	Nakajima and, Sorimachi 1995.
Largemouth bass *Micropterus salmonides*		North America	Plumb *et al*., 1996.
Sea bass *Lateolabrax sp.*		Japan (fish imported from Hong Kong)	Miyata *et al*., 1997.
Striped jack *Caranx delicatissimus*		South-East Asia#	Miyata *et al*., 1997.
Japanese parrot fish *Oplegnathus fasciatus*		Japan	Nakajima and Sorimachi 1995.
Spotted parrot fish *Oplegnathus punctatus*		South-East Asia#	Miyata *et al*., 1997.
Yellowtail *Seriola quinqueradiata*		Japan	Nakajima and, Sorimachi 1995.

HOST	**VIRUS#**	**ORIGIN**	*REFERENCE*
Amberjack *Seriola dumerili*		South-East Asia#	Miyata *et al*., 1997.
Goldstriped amberjack *Seriola aureovittata*		South-East Asia#	Miyata *et al*., 1997.
Brown-spotted grouper *Epinephelus tauvina*	SGD	South-East Asia (Singapore)#	Chua *et al*., 1994.
Brown-spotted grouper *Epinephelus malabaricus*		South-East Asia (Thailand)	Miyata *et al*., 1997.
Red spotted grouper *Epinephelus akaara*		South-East Asia#	Miyata *et al*., 1997.
Tiger puffer *Takifugu rubripes*		South-East Asia#	Miyata *et al*., 1997.
Guppy fish *Poecilia reticlata*		North America (U.S.A.) (fish imported from South-East Asia)	Hedrick and McDowell, 1995
Doctor fish *Labroides dimidatus*		North America (U.S.A.) (fish imported from South-East Asia)	Hedrickand McDowell, 1995.
Turbot Scophthalmus maximus		Europe (Denmark)#	Bloch *et al*., 1993.
Angelfish *Pterophyllum scalare*		North America (Canada)# (fish imported from unknown source)	Schuh and Shirley 1990.
Pike perch *Stizostedion lucioperca*		Finland	Tapiovaara *et al*., 1998.
Mudskipper *Parapocryptes serperaster*		Europe (Spain)# (fish imported from Malaysia)	Martinez-Picado *et al*., 1993.
Infectious spleen and Kidney necrosis virus	ISKV	China	He *et al*., 2002
AMPHIBIANS			
Leopard frog *Rana pipiens*	frog virus 3 (FV3) (type example of sympatric isolates FV1, 2, 9-23)	North America (U.S.A.)	Granoff *et al*., 1965.
Leopard frog *Rana pipiens*	LT1-LT4	North America (U.S.A.)	Clark *et al*., 1968.
Red eft *Diemictylus viridescens*	T6-20	North America (U.S.A.)	Clark *et al*., 1969.
***North American bullfrog* Rana catesbeiana**	tadpole edema virus (TEV)	North America (U.S.A.)	Wolf *et al*., 1968.
Edible frog *Rana esculenta*	REIR	Europe (Croatia)	Fijan *et al*., 1991.
Cane toad *Bufo marinus*	GV	South America (Venezuela)	Zupanovic *et al*., 1998.
Common frog *Rana temporaria*		Europe (U.K.)	Drury *et al*., 1995.
Red-legged frog *Rana aurora*	276	North America (U.S.A.)	Mao *et al*., 1997.
Tiger salamander *Ambystoma tigrinum* stebbensi	ATV	North America (U.S.A.)	Janovich *et al*., 1998.
REPTILES			
Box turtle *Terrapene c. carolina*	TV3	North America (U.S.A.)	Mao *et al*., 1997.
Central Asian *tortoise Testudo horsefieldi*	TV5	North America (U.S.A.)	Mao *et al*., 1997.
Gopher *tortoise Gopherus* polyphemus		North America (U.S.A.)	Westhouse *et al*., 1996.
Testudo *hermanni ranavirus*	ThRV		Marschang *et al*., 1999

* Australian ranaviruses not included; #, nomenclature as per cited reference.

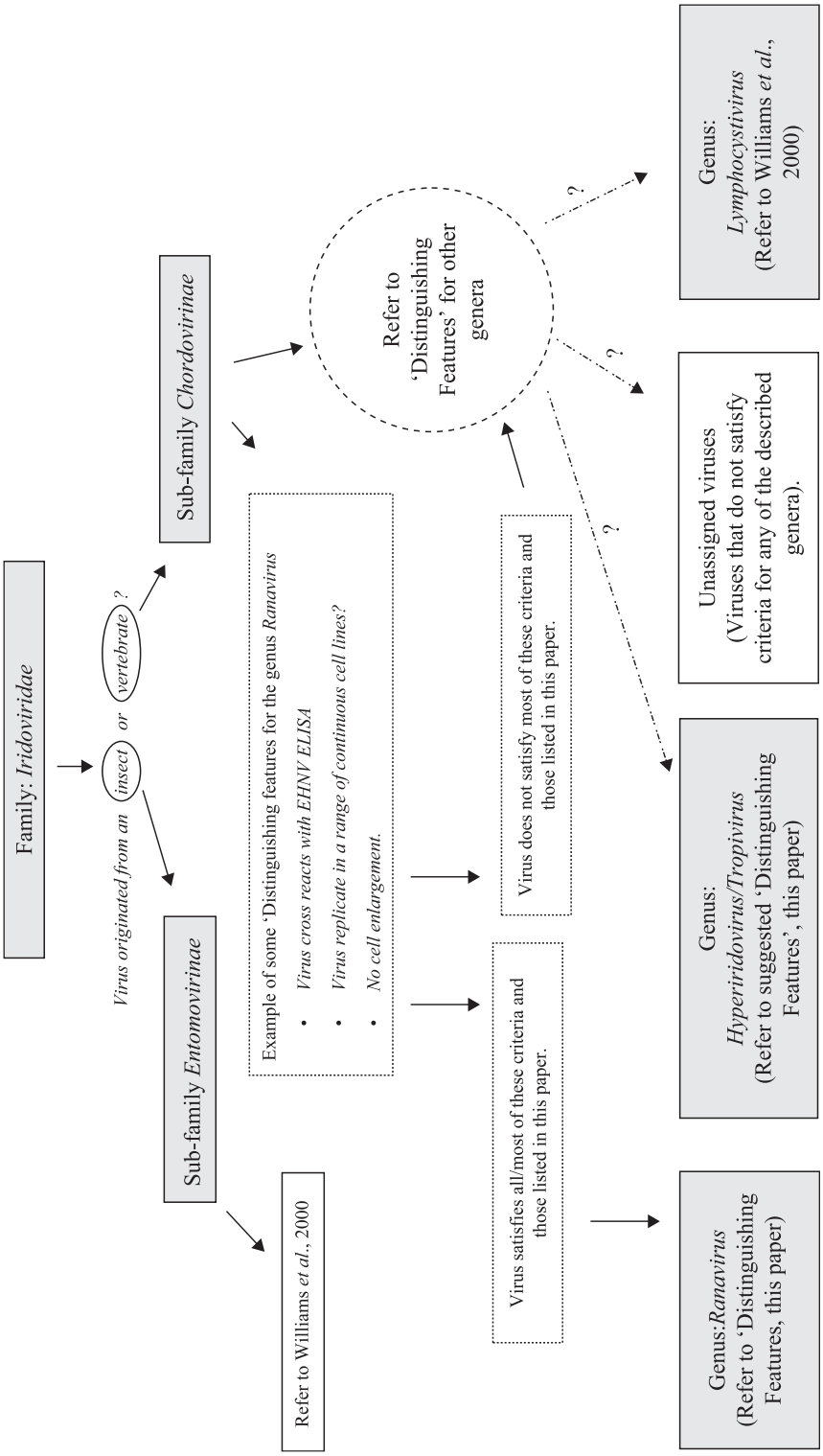

Figure 1. Schematic of suggested strategy for the classification of ranaviruses (based on Table 3). Schematic is shown to the level of genus.

The identification of many putative ranaviruses in one region of Northern America (e.g. Green *et al*., 2002) raises questions such as why are we now identifying these viruses and what has happened (eg anthropogenic changes) to cause the emergence of these viruses? To answer these questions it is critical that we can accurately identify these new viruses in terms of genera, species and genotypes. The ability to identify and differentiate ranaviruses will provide the background knowledge so that it may be possible to state whether a specific population of viruses is present/absent from any one region or country and whether a specific virus is increasing its range (issues in trade and conservation). It will also provide the basic knowledge to initiate research activities into attempting to answer the topical questions referred to in the Introduction.

Biology and Taxonomy of Ranaviruses

Identification of a ranavirus infection is based upon pathology and a battery of diagnostic assays including cell culture, ultrastructure/morphogenesis, (electron microscopy), antigenic analyses (ELISA, histochemistry, immunoelectron microscopy), SDS-PAGE, restriction endonuclease digestion, hybridisation, PCR analyses and sequencing (e.g. Hyatt *et al*., 2000). The data from all of these assays should be used in the overall classification and identification of any putative ranavirus. To explain the significance of this statement each of the above areas will be discussed in reference to categorising a virus to a specific level of classification. In addition a classification strategy for the identification of ranaviruses will be suggested.

Requirement for redefining iridovirus classification

The genus *Ranavirus* (refer below) contains a large group of viruses identified from fish, amphibians and reptiles. The many viruses described have differences in pathology, protein profiles, restriction fragment polymorphisms, antigenicity and sequence (refer to references in Table 2). That is, this group of viruses has become a large 'holding bag' for all 'iridoviruses' isolated from poikilothermic animals (excluding invertebrates), are not 'erythrocytic' or belong to the genus *Lymphocystivirus*. If we are to suggest a reclassification scheme for the vertebrate iridoviruses, specifically the ranaviruses, we should begin at the level of 'Family'.

Currently the ICTV classifies the family *Iridoviridae* into the genera, *Iridovirus, Chloridovirus, Lymphocystivirus* and *Ranavirus* (Williams *et al*., 2000). An outline and schematic of a proposed strategy for the classification of ranaviruses is shown in Fig. 1 and Table 3. Table 3 highlights a major change in the classification of iridoviruses with the introduction of 'sub-families'. The suggested classification strategy includes criteria based on either universal or polythetic classes and takes into consideration replicative lineage and ecological niches for species definition. An example of using an universal selection criterion is the presence/absence of methylated genomes. That is, this characteristic may be used to divide the family *Iridoviridae* into two sub-families namely *'Methylated Iridoviruses'* (including the current genera *Ranavirus* and *Lymphocystivirus*) and *'Non-methylated Iridoviruses'* (including the current genera *Chloridovirus and Iridovirus.*). Alternatively, the families could be divided into sub-families *Entomovirinae* and *Chordovirinae* as for poxviruses (Moyer *et al*., 2000). If consistency in the taxonomy of viruses is to be achieved then the taxonomy of iridoviruses should follow that existing for the more closely related families. As such, we suggest (based on the viruses listed by the ICTV) that the names of

the sub-families be *Entomovirinae* (non-methylated iridoviruses) and Chordovirinae (*methylated iridoviruses*); this scheme would have the advantage of accommodating other future sub-families encompassing other major groups of invertebrates.

Table 3. Suggested classification strategy for vertebrate iridoviruses.

Family:	Nature of genome & structure of virus (If a virus has a genome which is a single, linear dsDNA molecule (140-303 kb) that is circularly permuted and terminally redundant, in addition to having an icosahedral symmetry from 120 to 200nm then it belongs to the family *Iridoviridae*).
Sub Family:	Virus originates from insects (non-methylated) or vertebrates (methylated) (Classifies viruses into two groups e.g. *Entovirinae or Chordovirinae*[#].)
Genus*:	Distinguishing Features. (Polythetic class: properties in common, no single or set of defining properties). (Classifies viruses into genera e.g. *Chloriridovirus, Iridovirus, Ranavirus, Tropivirus, Hypervirus or Unassigned)* • Morphology • Physiochemical and Physical Properties • Nucleic acid • Lipid • Genome organisation and Replication • Antigenic Properties (e.g. PAGE, ELISA cross-reactivity. • Biological Properties (e.g. Cell and tissue tropism. Pathogenicity and cytopathology.)
Species:	Specific biological and genome properties. (Polythetic class: properties in common, no single or set of defining properties). (Classifies viruses into species within any one genus) • Genome sequence relatedness. • Natural host • RFLPs
Genotype:	Genome properties. • RFLPs • Multilocus sequencing

* Refer to Williams *et al*., 2000 for details. # Other Sub-Families could be created (as required) based on newly discovered or comprehensive characterisation of other 'iridoviruses'.

Requirement for the definition of new genera

As inferred above, the genus *Ranavirus* contains many viruses that require further description of their structure and function. Before we proceed we should briefly discuss the usage of this latter phrase. The phrase 'structure and function' is a complex one and is used to cover both the structure and function of the genome (i.e. sequence and properties/function of the encoded genes) and the ultrastructure of the virus and the collective functions of the virion in infection and subsequent disease. These characteristics can be identified as genome (sequence), encoded proteins (identification and function), antigenic properties (cross-reactivity), replication/morphogenesis, pathogenicity, natural host range, mode of transmission and physiochemical properties.

Selection of demarcation criteria for new genera

To divide the current ranaviruses into more appropriate groups (e.g. genera and species) we must define a list of 'Distinguishing Features' (demarcation criteria) for each level of classification. For a virus to be classified into any one group then it must satisfy most (but not necessarily all) of the defined properties for that group. To illustrate how this classification scheme would work we will take a closer look at the current group of ranaviruses. For example, there are two obvious groups of ranaviruses. One group is restricted to tropical fish, is associated with splenomegaly, anaemia, swollen kidneys, "highly ballooned cells" and little to no cross-reactivity to EHNV and FV3 (Sudthongkong *et al*., 2002). The second group includes the FV3-like viruses that are found in a range of fish, reptiles and amphibians, is not associated with the development of hypertrophied cells and cross-reacts in the EHNV antigen capture ELISA (Hyatt *et al*., 2000). Table 4 illustrates how these two groups can be objectively grouped into two different genera using seven demarcation criteria and a selection of viruses. We suggest that the name *Ranavirus* be retained as it is entrenched within the scientific literature. We also suggest that the second genus be called either *Hyperiridovirus* or *Tropivirus*. Sudthongkong *et al*. (2002) suggested that the name *Tropivirus* be used as it is representative of the geographical area from which all viruses that can be phylogentically grouped originated. An alternative name could be *Hypervirus* or indeed any another name that would represent the common property of this group namely the excessively large nature of the inclusion bodies (virus assembly sites) of the infected cells that contribute to their hypertrophic appearance. At this point in time, we shall refer to this second genus as *Hyperiridovirus* as it is a descriptive term that represents a common biological characteristic of this group.

From this analysis EHNV, BIV, European sheatfish virus (ESV) and doctorfish virus (DFV) are grouped into the genus *Ranavirus* whereas Red sea bream iridovirus (RSIV) satisfied only a few of the listed demarcation criteria for the same genus. For example, examination of the scientific literature (e.g. Inouye *et al*., 1992; Sudthongkong *et al*., 2002) reveals that RSIV is associated with marine fish, does not cross-react with antibodies against EHNV, requires a different set of PCR primers, has a 44% identity with FV3 and generates 'hypertrophied' cells. An analogous set of demarcation criteria could be generated to group the viruses similar to RSIV. Here differentiating criteria within "Distinguishing Features" (refer to Fig. 4) could include (i) 'viruses do not cross-react with the EHNV antigen capture ELISA but cross-react with polyclonal antibodies against RSIV' ('Antigenic Properties'); (ii) 'infected cells are enlarged due to the presence of large inclusion bodies/assembly bodies' ('Biological Properties') and (iii) viruses do not replicate in a range of continuous amphibian piscine and mammalian cell lines' ('Biological Properties'). If these criteria were used then RSIV would be included into the genus *Hyperiridovirus.*

An important point in the taxonomy of ranaviruses is that classification at the level of genus cannot be done on molecular biology alone. The reason for this is that unless the entire genome is sequenced and compared for numerous isolates then such taxonomy is of limited value. For example, the function of the genome comes from its structure; the varying biological properties that influence replication, virulence, host animals and transmission is a consequence of the structure of the genome; what may appear as inconsequential sequence differences may result in a significant biological phenotypic property (biological

characteristic). This is not to underestimate the importance of molecular biology. Sequence data is extremely important but it must be placed into context. It is important in determining the replicative lineage of viruses and increases in significance as the resolution of taxonomy (i.e. from species to genotype) increases.

Table 4. Suggested demarcation criteria for identifying viruses belonging to the genus ***Ranavirus.***

Genus: *Ranavirus* (Type species FV3)					
	EHNV	ESV	DFV	RSIV	BIV
1. >60 aa identity of MCP of type species.	Y	Y	Y	N	Y
2. Systemic and necrotising infection#	Y	Y	(?)	N	Y
3. Acquire plasma membrane and bud.	Y	Y	Y	N	Y
4. Cytoplamic assembly bodies, no hypertrophy/cytomegally.	Y	Y	Y	N	Y
5. Host range: Fish/amphibians/reptiles (one or more).	Y	Y	Y	Y	Y
6. Replicate in a range of continuous cell lines (including amphibian, piscine and mammalian)	Y	Y	Y	N	Y
7. Reactivity in EHNV antigen-capture ELISA*.	Y	Y	N	N	Y
Total	**7/7**	**7/7**	**5(?)/7**	**1/7**	**7/7**
Conclusion: EHNV, BIV, ESV & DFV are members of the genus: *Ranavirus.*					

aa: amino acid; (?), data not available; # pathology may also include haemorrhage and/or ulcers; *refer to Hyatt *et al*., 2000. Note, information relating to each of the criteria originates from the scientific literature cited in Table 2.

Selection of demarcation criteria for species, genotypes and importance thereof

As stated above it is important from trade, animal health and biological viewpoints to have the capacity to identify and differentiate species and genotypes within the current genus *Ranavirus*. For example, EHNV is listed by the OIE as a list B pathogen for the disease epizootic haematopoietic necrosis (EHN). Other viruses recognised by the OIE in association with EHN are ESV and ECV. This raises several questions namely, is EHNV a member of the species FV3, are redfin perch virus and rainbow trout virus (isolates of EHNV) different to each other and are ESV and ECV European isolates of EHNV? To answer these questions demarcation criteria must be listed for proposed species within the genus.

Demarcation criteria for 'species'. To date the ICTV recognises one species (FV3) within the existing genus Ranavirus. Within this species are listed the following isolates, box turtle 3 (TV3), Lucke titurus virus 1, tadpole edema virus and tortoise virus 5. Tentative species within the genus include BIV, EHNV (rainbow trout and redfin perch virus), Redwood Park virus (tadpole virus2, stickleback virus), Regina ranavirus (tiger salamander virus, Ambystoma tigrinum stebbinsi virus) and Santee-Cooper ranavirus (Largemouth bass virus, doctor fish virus, guppy virus 6).

Table 5. Suggested demarcation criteria for differentiating species within the genus *'Ranavirus'.*

Species demarcation criteria				
	EHNV	BIV	ESV	DFV
1. MCP gene and one other gene	Y	Y	Y	Y
(or part of) are different (e.g. 2%*) with other viruses within the genus.				
2. Specific natural host.	Y	Y	Y	(?)
3. RFLP bands differ significantly to other viruses (species) within genus (e.g. 20-30% bands in common). Viruses within same genus should share approximately 70-80% bands).	Y/Y	Y/(?)	Y/(?)	Y/(?)
	4/4	3/4	3/4	1/4
Conclusion: EHNV, BIV & ESV are distinct species. There is insufficient data to categorise DFV as a separate species.				

*Refer to Hyatt et al. (2000); (?) insufficient data. Note, information relating to each of the criteria originates from the scientific literature cited in Table 2.

Table 5 lists 4 demarcation criteria for the species FV3. In this table the viruses EHNV, BIV, ESV and DFV have been included for comparison. The analyses illustrates that EHNV is an individual species. If these criteria are used to analyse the data from Hyatt et al. (2000) then it can be proposed that there are currently five species within the current genus Ranavirus namely (i) FV3, (ii) ESV, (iii) DFV, (iv) BIV and (v) EHNV.

Are all members of a single species isolates of the one 'population'? It is generally accepted that members within a group such as 'species' are 'plastic'. That is, due to evolutionary pressures there is some variation in the structure and function of the genome. Therefore, without the use of differentiating neutralising antibodies and access to sequence data of complete genomes or validated portions thereof, how can we identify distinct ranavirus populations within a species (i.e. identify different genotypes)? Most diagnostic assays provide data pertinent to identifying viruses to the levels of genus and on occasion, species. For example, SDS-PAGE identifies polypeptide profiles indicative of a specific genus and the presence of a major 48 to 52 kDa MCP (Hyatt *et al*., 2000). Antigen capture ELISAs (and other antigen - based assays) illustrate cross-reactivity with most viruses at the genus level, and ultrastructure, which as a 'rule of thumb' provides general information

on replication and cytopathology useful at the genus level. It should, however, be noted that subtle ultrastructural differences can be observed in ultrastructural pathology (manifestations of changes in genomic structure) which are indicative of specific species (e.g. Wamena virus, Hyatt *et al*., 2002). Collectively, these diagnostic assays cannot differentiate between distinct species or between distinct populations within anyone species.

Within this paper, genotypes are defined as sub-populations of a species that generate progeny virions of high fidelity (maintenance of the specific genomic structure and function). To identify such genotypes high-resolution diagnostic assays are required. These analytical tools should examine the fidelity of the entire genome. To test fidelity many isolates should be analysed as a function of time, host(s) and geographical range. If fidelity is conserved then assumptions in relation to the taxonomic status of future new isolates can be made (i.e. the use of a set number of genes or part there of). At present only a few methylated iridovirus genomes have been sequenced in their entirety; key genes to define specific species and genotypes have therefore yet to be identified. Alternatively, restriction endonuclease digestion of complete genomes can be performed. To increase the sensitivity of this technique a minimum of three enzymes should be used.

As one of the suggested demarcation criteria for identification of a species is that all members should have a minimum of 60% to 80% RFLP bands between similar isolates we suggest that genotypes should display greater than 80% homology. Using this approach we can challenge inferences that there are two currently identified genotypes of EHNV namely rainbow trout virus (RTV) and redfin perch virus (RFPV) (Williams *et al*., 2000). The RFLPs of various EHNV isolates collected over different time periods, geographical ranges and the two different hosts (Hyatt *et al*., 2000) indicate that the isolates are very similar, i.e. whilst there are differences between the isolates, these differences appear random and cannot be explained on the basis of host animal.

The use of an objective, logical taxonomic strategy can therefore be used to demonstrate that EHNV is a distinct species. Furthermore the data from Hyatt *et al*. (2000) suggests that there are currently five species within the current genus *Ranavirus* namely (i) FV3, (ii) ESV, (iii) DFV, (iv) BIV and (v) EHNV. The data also suggests that of the many isolates of EHNV so far characterised there are no distinct genotypes (correlation of RFLPs with disease characteristics).

Summary

Over the past ten to fifteen years many viruses have been identified from Australian poikilotherms. Of these, ranaviruses are the only viruses that increased their geographical range. This genus of viruses is distinct from the group of viruses isolated from tropical fish that are associated with hypertrophied cells. Ranaviruses have been identified from many other countries including North and South America, Europe and Asia. Whilst pathogenic to many animals, and probably representative of many of their natural viral assemblage, this group of viruses has not been associated with long-term population declines. They are however, identified by the OIE and are recognised as potential threats to aquaculture and free-ranging animals.

The current taxonomy of 'ranaviruses' is in need of revision. We have discussed what we believe to be the shortcomings and have suggested a more complex and rigorous classification scheme. From such an approach we suggest that the family *Iridoviridae* be divided into two sub-families *Entomovirinae* and *Chordovirinae.* Within the sub-family *Chordovirinae* we suggest that (i) the genus *Ranavirus* be retained (i.e. not renamed) and (ii) at least one other genus be created (eg. *Hyperiridovirus or Tropivirus*) to include the tropical viruses that are associated with the development of hypertrophied cells. We further suggest that a list of demarcation criteria be established for the identification of specific genotypes which should decrease confusion about identity of specific viruses, i.e. is a newly identified virus an isolate of an existing species and genotype or is it a genuinely new virus constituting a new species or new genotype of an existing species? Finally, with the implementation of a new methodical approach to the taxonomy of ranaviruses meaningful research into topical questions referred to in the 'Introduction' can be initiated.

Note: Since the writing of this manuscript the International Committee on the Taxonomy of Viruses (ICTV) have accepted the naming of the genus referred to within this paper as "*Hyperiridovirus*" or "*Tropivirus*" as "*Meglaocytivirus*" from the Greek meaning "enlarged cell".

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