



Diversity and classification of reoviruses in crustaceans: A proposal

Mingli Zhao^a, Camila Prestes dos Santos Tavares^b, Eric J. Schott^{c,*}

^a Institute of Marine and Environmental Technology, University of Maryland, Baltimore County, Baltimore, MD 21202, USA

^b Integrated Group of Aquaculture and Environmental Studies, Federal University of Paraná, Rua dos Funcionários 1540, Curitiba, PR 80035-050, Brazil

^c Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, MD 21202, USA

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ABSTRACT

A variety of reoviruses have been described in crustacean hosts, including shrimp, crayfish, prawn, and especially in crabs. However, only one genus of crustacean reovirus - *Cardoreovirus* - has been formally recognized by ICTV (International Committee on Taxonomy of Viruses) and most crustacean reoviruses remain unclassified. This arises in part from ambiguous or incomplete information on which to categorize them. In recent years, increased availability of crustacean reovirus genomic sequences is making the discovery and classification of crustacean reoviruses faster and more certain. This minireview describes the properties of the reoviruses infecting crustaceans and suggests an overall classification of brachyuran crustacean reoviruses based on a combination of morphology, host, genome organization pattern and phylogenetic sequence analysis.

1. Introduction

1.1. Genera of Reoviridae family

Reoviridae is the largest and the best studied family of all the double-stranded RNA (dsRNA) virus families (Mertens, 2004). Viral particles of reoviruses have icosahedral symmetry, with diameters of 55–85 nm and can be subdivided into two subfamilies, the ‘turreted’, and ‘non-turreted’ viruses, depending on whether they have projections at the 12 vertices (Attoui et al., 2012). Reovirus genomes are composed of segmented dsRNA; the number of genome segments (9, 10, 11 or 12) is characteristic of viruses within a single genus (Attoui et al., 2012). In addition to the structural classification, the level of sequence divergence, particularly in the more conserved genome segments and proteins, are important criteria for the classification of genera and species. For example, amino acid identities of RNA dependent RNA polymerase (RdRps) within a single genus are usually >33% (Lefkowitz et al., 2018). In total, 97 species in the *Reoviridae* family have been classified into 15 genera and divided between two subfamilies. The subfamily *Spinareovirinae* (turreted) contains 9 genera: *Aquareovirus*, *Coltivirus*, *Cypovirus*, *Dinovernavirus*, *Fujivirus*, *Idnoreovirus*, *Mycoreovirus*, *Orthoreovirus* and *Oryzavirus*. The subfamily *Sedoreovirinae* (non-turreted) includes 6 genera: *Rotavirus*, *Phytoreovirus*, *Orbivirus*, *Seadornavirus*, *Mimoreovirus* and *Cardoreovirus* (Walker et al., 2019). Reoviruses have been isolated from a wide range of host species, including mammals, birds, reptiles,

fish, crustaceans, marine protists, insects, ticks, arachnids, plants and fungi (Attoui et al., 2005; Shields et al., 2015). Host range and disease symptoms are also important indicators that help to identify viruses of different genera (Attoui et al., 2012).

1.2. Reoviruses in aquatic hosts

The *Reoviridae* family contains four distinct groups of aquatic reoviruses, two of which infect teleost fish: *Aquareovirus* with 11 genome segments (Lupiani et al., 1995; Attoui et al., 2012), and isolates of the newly discovered piscine orthoreovirus (PRV) with 10 genome segments in the genus *Orthoreovirus* (Palacios et al., 2010; Godoy et al., 2016; Kibenge and Godoy, 2016). The *Micromonas pusilla* reovirus (MprV) has 11 genome segments, infects a picophytoplankton species, and is the founding member of the genus *Mimoreovirus* (Brussaard et al., 2004). The only ICTV-recognized genus of crustacean reoviruses is *Cardoreovirus* with 12 genome segments and infects Chinese mitten crab *Eriocheir sinensis* (*E. sinensis*) (Zhang et al., 2004; Attoui et al., 2012). However, since the first discovery of a reo-like virus from a marine crab *Macropipus depurator* (*M. depurator*) in 1966 (Vago, 1966), diverse reoviruses have been reported in crustacean hosts, particularly in *Portunidae* and *Varunidae* crabs, as well as other crustaceans such as crayfish, shrimp and prawn (Johnson, 1984; Bateman and Stentiford, 2017; Shields et al., 2015). The classification of these crustacean reoviruses is less well developed than reoviruses of vertebrates, in part because of the

* Corresponding author.

E-mail address: schott@umces.edu (E.J. Schott).

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Table 1
Properties of reoviruses described in crabs. The genus of crab reoviruses was based upon previous descriptions and phylogenetic analysis in this review.

Host	Reovirus name	Genus	Virion size (nm)	Viral Inclusion morphology	RNA segment	RNA gel pattern	Seq- uence	Genome size (nt)	Infection sites	Clinical signs	References
<i>Macropis depurator</i>	Reovirus	NA	50–60	paracrystalline	NA	NA	NA	NA	hemocytes and connective tissue;	paralysis;	Vago (1966), Bonami (1973), Bonami et al. (1976)
	P virus	Crab-	~64	paracrystalline	12	1/5/6	E2 (700 bp)	NA	hemocytes and connective tissue;	paralysis and mortality;	
<i>Carcinus mediterraneus</i>	W2 virus	Crab-	65–70	rosettes	12	1/5/6	NA	NA	hemocytes and connective tissue;	mortality;	Mari and Bonami (1986), Mari and Bonami (1986)
	RC84		70–75	paracrystalline (occasional)	NA	NA	NA	NA	B cells of hepatopancreas;	NA	
<i>Callinectes sapidus</i>	RLV	Crab-	~55	paracrystalline	NA	NA	NA	NA	hemocytes and connective tissue;	paralysis, abnormal feeding, anorexic lethargic, mortality;	Johnson (1977)
	CsRV1	Crab-	~55	paracrystalline	12	1/5/6	genome	23,913	hemocytes and connective tissue;	lethargic, mortality;	Bowers et al. (2010), Zhao et al. (2021)
<i>Scylla serrata</i>	CsRV2	Crab-	NA	NA	12	3/4/2/3	genome	NA	NA	NA	
	MCRV	Crab-	70	paracrystalline	12	1/5/6	genome	24,464	connective tissue;	“SD”: lethargic, anorexic, mortality;	Weng et al. (2007)
	SsRV	Crab-	70	paracrystalline	12	1/5/6	genome	24,464	connective tissue;	“CD”: mortality;	Zhang et al. (2007), Chen et al. (2011)
<i>Eriocheir sinensis</i>	EsRV905	Crab-	55	NA	12	3/4/2/3	RdRp	NA	connective tissue;	30% mortalities, no trembling signs;	Zhang et al. (2002), Zhang et al. (2002), Shen et al. (2015)
	EsRV816	Crab-	~60	NA	10	5/3/2	RdRp	NA	connective tissue;	NA	
	EsRV WX-2012	Crab-	60–70	paracrystalline	12	1/5/6	genome	23,913	NA	NA	
<i>Portunus trituberculatus</i>	SCRV	NA	30 ± 10	NA	NA	NA	NA	NA	hemorrhaging and mortality		Li et al. (2012)

Note: “NA” refers as no data available.

diversity of hosts, and lack of cell culture methods for propagation. In some cases, the lack of viral genome sequences upon first discovery resulted in different names being given to the same virus. With more genomic and metagenomic sequences of crustacean reoviruses becoming available, now is a good time for a taxonomic overview of brachyuran crustacean reoviruses. In this minireview, we present a comprehensive overview of reoviruses recorded in crustaceans, host ranges, geographic distribution and pathology. We emphasize the diversity and classification of brachyuran crustacean reoviruses based on their genome organization pattern and sequence phylogenetic analysis. From these lines of evidence, we propose a hypothetical classification of reoviruses in brachyuran crabs that clarifies some of the ambiguities in their naming.

2. Reoviruses in brachyuran crustaceans

2.1. Host range and geographic distribution

Fourteen reoviruses have been reported from seven crab species within *Portunidae* and *Varunidae* families along the coasts of Asia, Europe, North America and South America (Table 1; Fig. 1). In fact, the first virus discovered in a marine invertebrate was a reo-like virus from *M. depurator* on the French coast of the Mediterranean (Vago, 1966). Bonami (1973) described a virus infecting *M. depurator* in the same area and designated it as reovirus based on its morphological characteristics and named as P virus (P for paralysis). Both viruses have similar viral particles with a size of ~60 nm forming paracrystalline arrays in the connective tissue and hemocytes (Vago, 1966; Bonami et al., 1976). At the French Mediterranean coast near Seté, two reoviruses (W2 and RC84) were observed in diseased shore crabs *Carcinus mediterraneus* (*C. mediterraneus*) (Mari, 1987; Mari and Bonami, 1986; 1987; 1988). W2 was named based on its ultrastructural similarities with W virus, which has little available information except that it infects *Carcinus maenas* (Bonami and Zhang, 2011). W2 virus is 65–70 nm in diameter and forms rosettes in connective tissue of the hepatopancreas, digestive tract, gills and hemocytes, while RC84 has paraspherical 70–75 nm virions, infecting B-cells and R-cells of the digestive epithelium of the hepatopancreas (Mari and Bonami, 1986; 1987; 1988).

At the same time that P virus was discovered in the Mediterranean, a pathogenic reovirus (RLV = reo-like virus) was identified in *Callinectes sapidus* (*C. sapidus*) on the other side of the Atlantic Ocean (Johnson and Bodammer 1975; Johnson, 1977). A similar reovirus was discovered in *C. sapidus* captured from the Chesapeake Bay in 2009 and was present in >50% of dead or dying soft-shell crabs but fewer than 5% of healthy hard crabs (Bowers et al., 2010; Spitznagel et al., 2019), and designated as *Callinectes sapidus* reovirus (CsRV) (Tang et al., 2011). These cytoplasmic viruses with icosahedral capsid are ~55 nm in diameter (Fig. 2). CsRV was renamed as CsRV1 (Flowers et al., 2016a; 2016b; 2018; Zhao et al., 2020) when a second reovirus was discovered in *C. sapidus* collected from southern Brazil and provisionally named CsRV2 (Fig. 3A) (Zhao et al., 2021). CsRV2 was discovered solely by the presence of its dsRNA genome in RNA extracts from *C. sapidus*, and nothing else is known about its potential pathogenicity or prevalence in this host. More recently, CsRV2 was discovered infecting *Callinectes danae* (*C. danae*) in Brazil and is the subject of ongoing investigations (Tavares et al., unpublished).

Half of the brachyuran crustacean reoviruses have been identified infecting aquaculture crabs in China, including MCRV and SsRV infecting mud crab *Scylla serrata* (*S. serrata*) (Weng et al., 2007; Zhang et al., 2007; Ma et al., 2016); EsRV905, EsRV816 and EsRV WX-2012 identified in *E. sinensis* (Bonami and Zhang, 2011; Shen et al. 2015; Zhang et al., 2002; Zhang and Bonami, 2012) and SCRv infecting *Portunus trituberculatus* (*P. trituberculatus*) (Fang et al., 2015; Zhang et al., 2015). The viral particle of both MCRV and SsRV is 70 nm, icosahedral and nonenveloped (Weng et al., 2007; Chen et al., 2011). Deng et al. (2012) suggested MCRV belongs to a new genus of the *Reoviridae* family,

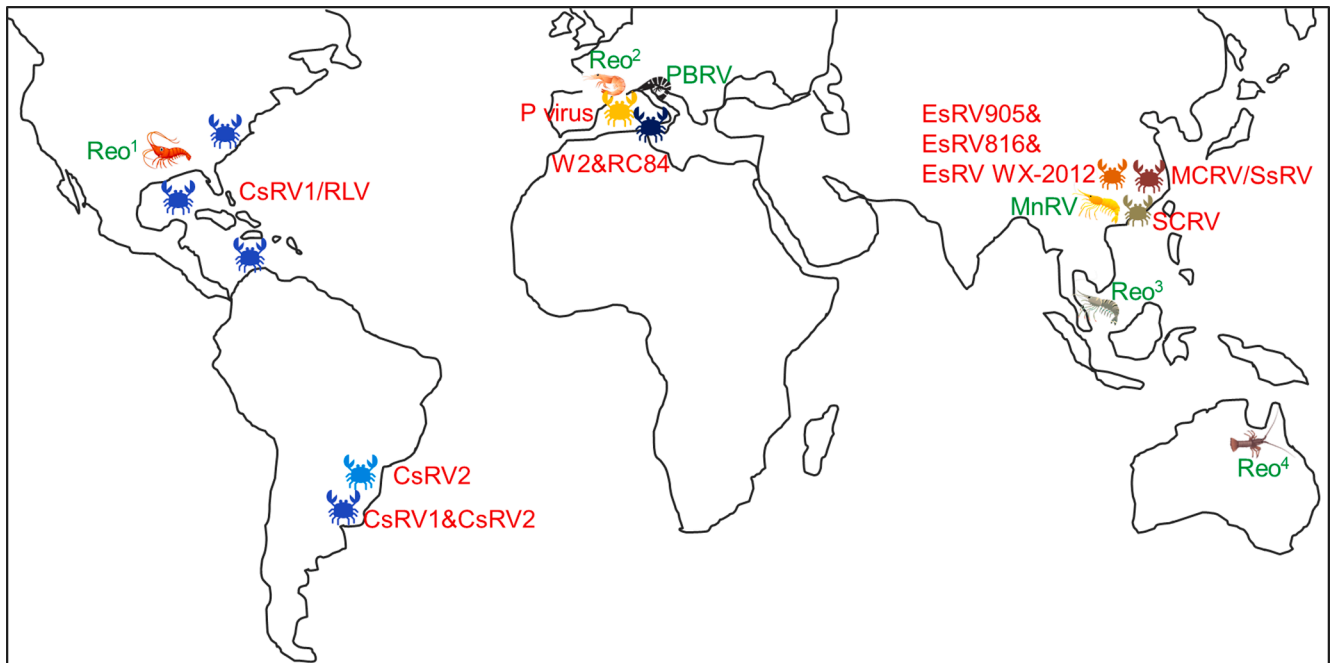


Fig. 1. Geographic distribution and host ranges of crustacean reoviruses. Reoviruses infecting crabs are shown in red and other crustaceans (shrimp, prawn and crayfish) in green. Cartoon images are obtained from <https://ian.umces.edu/>. Note: RLV is a specific name for the reovirus infecting *C. sapidus* (same to CsRV/CsRV1); Reo¹, Reo², Reo³, Reo⁴ are used here only for specifying that these are different reo-like virus.

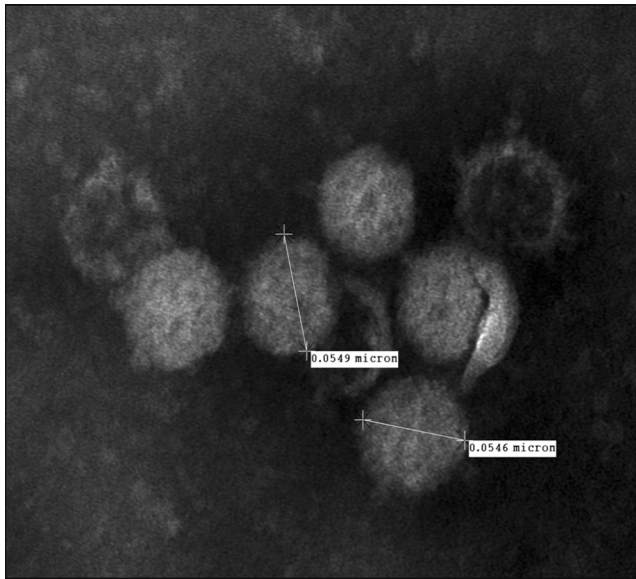


Fig. 2. Electron microscopic image of CsRV1. The size of CsRV1 is ~55 nm. Muscle of an infected crab was homogenized, clarified by centrifugation, and filtered through 0.2 μ m filter. Virus particles were concentrated by centrifugation at 22,000g, suspended in Tris and adsorbed to Formvar/copper coated grids and stained with phosphotungstic acid. Samples were visualized using a FEI Tecnai TM T12 transmission electron microscope.

named *Crabreovirus*, which has yet to be recognized by ICTV. Both EsRV905 and EsRV816 were described in *E. sinensis* afflicted with “trembling disease” (TD) in southern China. Non-enveloped icosahedral viral particles infect connective tissue but of different diameters, 55 nm and ~60 nm for EsRV905 and EsRV816, respectively (Zhang et al., 2002; Zhang and Bonami, 2012). A third virus, EsRV WX-2012 was also identified from Chinese *E. sinensis* exhibiting “TD”; it has a typical reovirus icosahedral structure and a diameter of 60–70 nm (Shen et al.

2015). The swimming crab reovirus (SCRv) (Fang et al., 2015; Li, 2012; Li et al., 2012; Zhang et al., 2015) was isolated from *P. trituberculatus* in China. The complete SCRv particle is reported to be 30 ± 10 nm, which is smaller than most reoviruses (Zhang et al., 2015).

2.2. Pathology of reoviruses in brachyuran crustaceans

2.2.1. Clinical signs and mortality

The typical clinical signs caused by reovirus infections in brachyuran crustaceans are lethargy, anorexia, trembling and paralysis at late phases of the infection (Vago, 1966; Johnson, 1977; Bonami and Zhang, 2011). For most crab reoviruses, disease signs and mortality were reproduced in experimentally infected crabs through injection or oral inoculation (Bonami et al., 1976; Bonami and Zhang, 2011; Bowers et al., 2010; Mari and Bonami, 1988; Weng et al., 2007). Experimental injection of purified P virus caused trembling of 60% *M. depurator*, followed by paralysis and a mortality of 70–80% in 9 days (Bonami et al., 1976). W2 virus was detected in crabs 5 days after exposure and diseased crabs died within 20 days (Mari and Bonami, 1988). Injection of RLV/CsRV1 caused 100% mortality and resulted in the appearance of viral RNA and virus inclusions in hemocytes (Johnson, 1977; Bowers et al., 2010). CsRV1 was also present at high prevalence in a majority of wild crabs that died in soft-shell production systems (Spitznagel et al., 2019). Experimental infection of *S. serrata* with MCRV by intramuscular injection, bath inoculation and oral inoculation led to 100% mortality, while cohabitation caused 80% mortality (Weng et al., 2007). However, experimental infection of *E. sinensis* using purified EsRV905 or EsRV816 caused only 30% mortality without signs of trembling, and the virus could be detected in surviving crabs (Bonami and Zhang, 2011; Zhang et al., 2002). SCRv can provoke severe hemorrhaging in *P. trituberculatus* and causes up to 100% mortality (Zhang et al., 2015). No experimental infection has been conducted for RC84, EsRV WX-2012, CsRV2 and SsRV, but SsRV was discovered infecting *S. serrata* with “waterclear disease (CD)”, which caused ~80% mortality at the infected farms in Zhejiang province (Chen et al., 2008) (Table 1).

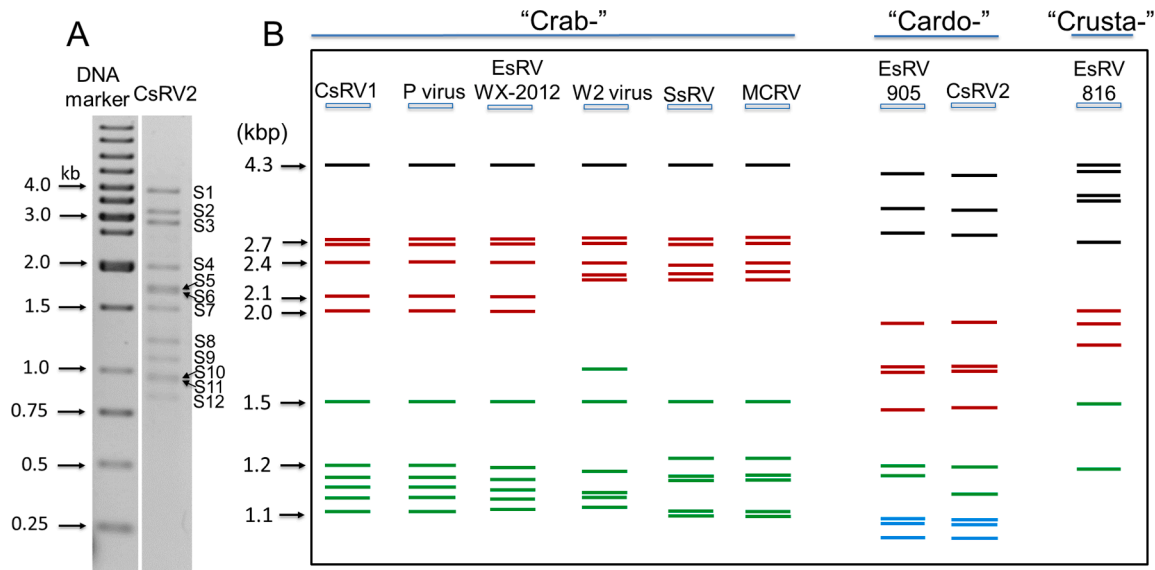


Fig. 3. Taxonomic grouping of reoviruses infecting brachyuran crabs based on agarose gel electrophoresis. A. Agarose gel electrophoresis of CsRV2 (Zhao et al., 2021); B. Schematic of the dsRNA banding patterns of all available crab reoviruses. Three different genera are suggested as “Crabreovirus”, “Cardoreovirus” and “Crustareovirus”.

2.2.2. Reoviruses infecting different hosts – “Cross infection”

Host range has been an important facet of reovirus characterization. Very few of the crustacean reoviruses have been tested for infectivity in species other than the original host. Experimental cross infection of P virus and W2 in their respective crab hosts was not successful: P virus did not replicate in *C. mediterraneus* and conversely W2 not replicate in *M. depurator* (Bonami, 1980; Mari, 1987). However, P virus has >98% nucleotide sequence identity with CsRV1 (GenBank accession no. MW088922; NC_037581) (Flowers et al., 2016), suggesting that these two viruses are likely different variants of a same reovirus infecting different crab species - *M. depurator* and *C. sapidus* in Mediterranean and Atlantic coasts, respectively. CsRV2, while first discovered in *C. sapidus*, appears to also infect *C. danae* in Brazil (Zhao et al., 2021; Tavares et al., unpublished). It has not been established whether CsRV2 is pathogenic to either *C. sapidus* or *C. danae*.

2.2.3. Hosts infected by different reoviruses - “Multi-infection”

It is not uncommon for the presence of two or more pathogens to be detected in wild or captive diseased crustaceans. Indeed, some of the early descriptions of the blue crab reovirus by Johnson (1984) included discussion of baculo- or rhabdo-like co-infections. Multi-infection of reoviruses in brachyuran crustaceans has also been verified in several crab species. *C. sapidus* was found to harbor two different reoviruses simultaneously- RLV/CsRV1 and CsRV2 (Bowers et al., 2010; Zhao et al., 2021). W2 and RC84 were both identified from the same crab species - *C. mediterraneus* (Mari and Bonami, 1986; 1987; 1988). EsRV905 and EsRV816 were isolated from the same study of *E. sinensis* afflicted with TD (Zhang et al., 2002). In 2005, a novel *E. sinensis* reovirus WX-2012 (EsRV) was also identified from cultured *E. sinensis* exhibiting TD (Shen et al., 2015). Co-infection of related segmented RNA reoviruses, such as CsRV1 and CsRV2, in a host cell could provide chances for viral reassortment, result in the shuffling of gene segments to generate progeny viruses with novel genome combinations. In addition, the likelihood of co-infection of reoviruses in crustaceans might result in cumulative pathogenicity of each virus and be a potential threat to crustacean aquaculture.

3. Classification of brachyuran crustacean reoviruses

3.1. Genome organization pattern

Except for EsRV816, which has a genome with 10 linear dsRNA segments (Zhang et al., 2002), all known brachyuran crustacean reoviruses have 12 segmented dsRNA genomes (no information is available for SCRv). The pattern (number and sizes) of genome segments is an informative criterion for the classification of reoviruses. According to their genome organization patterns based on either agarose gel electrophoresis or segments derived from genome sequences, the brachyuran crustacean reoviruses can be divided into 3 groups (Fig. 3B): 1) *Crabreovirus*: P virus, W2, EsRV WX-2012, CsRV1, MCRV and SsRV have a pattern of 1/5/6 (1 large, 5 medium and 6 small sized segments); 2) *Cardoreovirus*: EsRV905 and CsRV2 have a 3/4/2/3 pattern; 3) and *Crustareovirus*: the genome pattern of EsRV816 is 5/3/2 (Bowers et al., 2010; Chen et al., 2011; Chen et al., 2012; Deng et al., 2012; Flowers et al., 2016; Montanie et al., 1993; Shen et al., 2015; Weng et al., 2007; Zhang et al., 2004; Zhang and Bonami, 2012).

3.2. Genome-based phylogenetic analyses

Genome sequence comparisons are a powerful way to understand the relationships between related viruses. Whole genome sequences are available for CsRV1 (Flowers et al., 2016), CsRV2 (Zhao et al., 2021), MCRV (Deng et al., 2012), SsRV (Chen et al., 2011; Chen et al., 2012), and EsRV WX-2012 (Shen et al., 2015). The genome sizes of these reoviruses are all between 23 and 25 kbp (Table 1). The genome sequence of SsRV has 67 nucleotide variations compared to MCRV, of which only 11 result in amino acid changes. Thus, Chen et al. (2012) suggested the two pathogens are likely to belong to the same species. The genome of CsRV2 identified from *C. sapidus* was recently sequenced (Zhao et al., 2021). The P virus genome is largely un-described, except for a ~700 bp region used to construct a dot-blot probe and represents the first published sequence of a crab reovirus (Walton et al., 1999). Partial RdRp gene sequence is available for EsRV905 and EsRV816 (Zhang et al., 2004; Zhang and Bonami, 2012). No sequence data is publicly available for most of the reoviruses detected during the 1970s and the first half of the 1980s, such as the reovirus discovered by Vago (1966), W2, RC84 and RLV (Table 1).

The RdRp gene has been used to elucidate the evolutionary

relationships among viruses in the genera of the family *Reoviridae* because the polymerase enzyme is the most conserved of the viral proteins. To date, only EsRV905 has been officially accepted by ICTV in *Cardoreovirus* genus (Attoui et al., 2012), and all other crustacean reoviruses have not been assigned into any genus. Nevertheless, a phylogenetic tree could provide insights into the relationships of crab reoviruses to each other and to reovirus genera in other hosts. An alignment of protein sequences for RdRp shows that CsRV2 shares ~80% identity in RdRp amino acid sequences with EsRV905, and so appears to be a 2nd member of the *Cardoreovirus*. The remaining crab reoviruses cluster into two different clades (Fig. S1; Fig. 4). The most populated clade, *Crabreovirus*, consists of MCRV, SsRV, CsRV1 and EsRV WX-2012, which encode RdRp proteins that share >85% amino acid identity. Because the RdRp proteins encoded by MCRV and SsRV are >99% identical (Chen et al., 2012; Zhang et al., 2007), it is likely that these two viruses are variants of the same species.

The available RdRp amino acid sequences of EsRV816 shows less than 15% identity with members of the *Crab-* or *Cardoreovirus* genera. The RdRp of EsRV816 does however share 60% and 45% identity with two putative reoviruses described in a metagenomic analysis of crustaceans: Wenling Reo-like virus 1 (WLR1) and Beihai Reo-like virus 2 (BHR2) (Zhang and Bonami, 2012; Shi et al., 2016). These three sequences may therefore represent a new genus, which we propose a provisional name of *Crustareovirus* (Fig. 5). However, future studies of virus particle characteristics, host species, pathogenesis, and genome organization of WLR1 and BHR2 will be needed to support this classification (Shi et al., 2016).

Although no RdRp sequence of P virus is available for phylogenetic

analysis, Walton et al. (1999) reported 700 nt of the genome which was recognized later to be 97% identical to CsRV1 segment 4 (Flowers et al., 2016), suggesting that these two viruses could be variants of a same reovirus species. The size difference between P virions (Bonami, 1973) and CsRV1 (Fig. 2) could be caused by different purification protocols since the outer capsid proteins could have been lost or degraded during the centrifugation, or different measurement methods. Additionally, P virus and W2 virus have been suggested to constitute a genus, based on their dsRNA electrophoretic pattern (1/5/6) (Montanie et al., 1993) (Fig. 3B). Therefore, P virus and W2 virus could be added into the genus *Crabreovirus*, which extends the genus to 6 members (Fig. 5).

4. Reoviruses in other crustacean hosts: shrimp, crayfish and prawns

Reoviruses have been identified from 6 different species of shrimp, crayfish and prawn, including *Penaeus monodon* (*P. monodon*), *Penaeus japonicus* (*P. japonicus*), *Penaeus vannamei* (*P. vannamei*), *Palaemon elegans* (*P. elegans*), *Cherax quadricarinatus* (*C. quadricarinatus*), and *Macrobrachium nipponense* (*M. nipponense*) (Table 2). Most of these reoviruses were described prior to the development of molecular tools to make sequencing and analysis of virus genomes rapid and inexpensive, and partial genome sequences are available only for the reoviruses reported from *C. quadricarinatus* and *M. nipponense*.

4.1. Reovirus in Penaeidae

A reo-like virus, showing intracytoplasmic reoviral arrays comprised

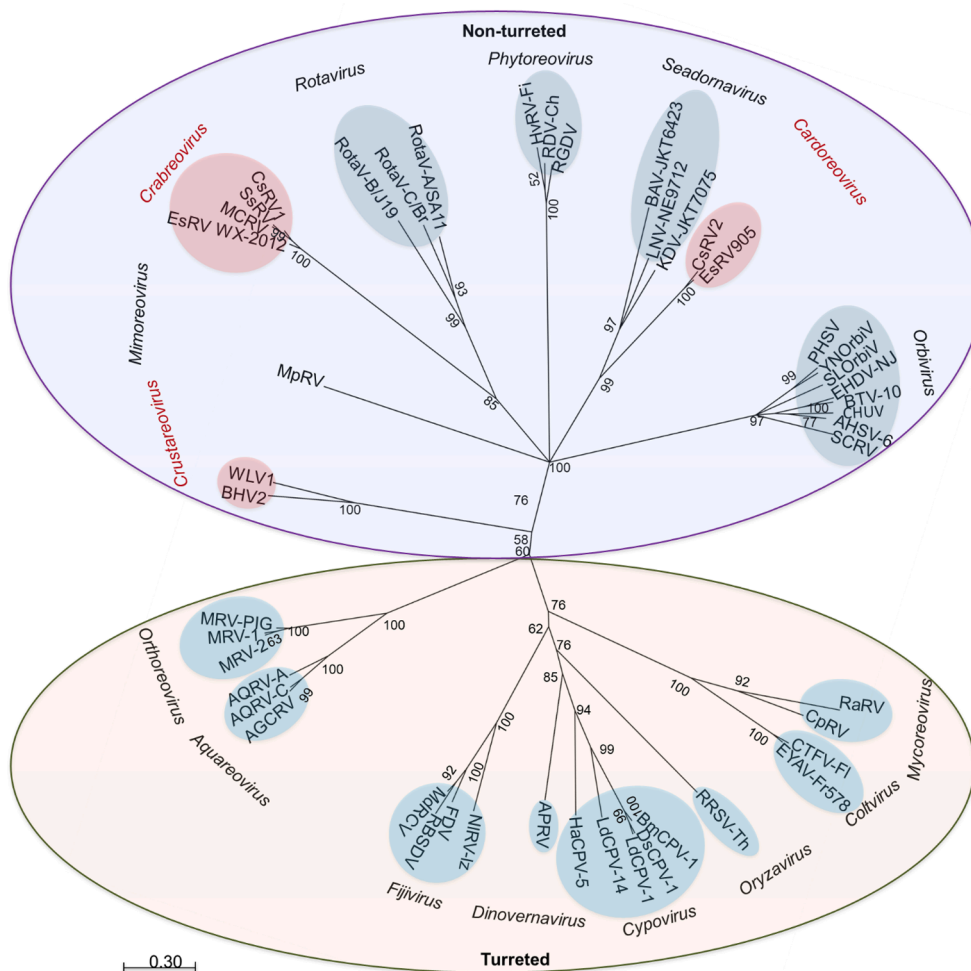


Fig. 4. Neighbor-joining phylogenetic tree of crustacean reoviruses and other reoviruses based on amino acid sequences of RdRp gene. Accession numbers and abbreviations are available in Supplementary Table S1. RdRp sequences were aligned and phylogenetic tree was constructed using CLC Workbench 7 (Qiagen). Bootstrap support with 1000 replicates is shown above the branches. The three genera of brachyuran crustacean reoviruses within *Reoviridae* family are shown in red: *Crabreovirus*, *Cardoreovirus* and *Crustareovirus*.

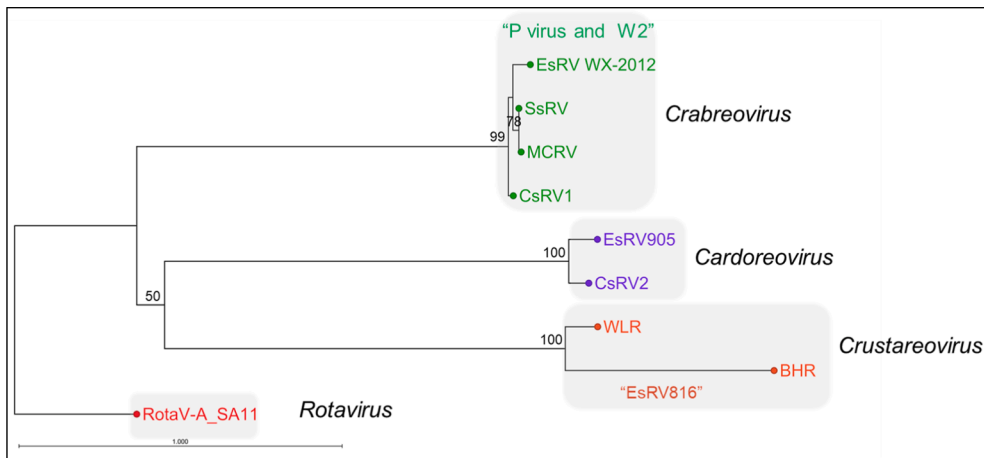


Fig. 5. Theoretical classification of brachyuran crab reoviruses based on phylogenetic analysis, genome organization pattern and sequence identity. The tree was drawn from subset of the RdRp alignment used in Fig. 4, and viruses for which no RdRp sequence data is available were added based on criteria listed in the text. Sequences were aligned and phylogenetic tree was constructed with CLC Workbench 7 (Qiagen). Bootstrap support with 1000 replicates is shown above the branches.

of non-enveloped icosahedral or paraspherical particles measuring 50–70 nm, was identified from moribund and dying juvenile *P. monodon* in Malaysia (Anderson et al., 1987). However, the pathogenesis of this reovirus was undetermined, since the *P. monodon* was co-infected with baculovirus, rickettsia and gram-negative bacterial (Nash et al., 1988). Tsing and Bonami (1987) discovered typical reoviral non-enveloped, icosahedral particles with a diameter of 61 nm in cultured *P. japonicus* experiencing mass mortalities. Virions were observed to develop in the cytoplasm of hepatopancreatic R-cells without any crystalline arrangement. This reovirus was thought to have limited pathogenicity as the virus can also be found in non-diseased shrimp (Lightner, 1988). A reo-like virus was found infecting *P. vannamei* concurrently with experimental infection of *Baculovirus penaei* (BP) (Krol et al., 1990). Both viruses occurred occasionally in the same epithelial cells of the anterior midgut and in R- and F-cells of the hepatopancreas. It is not clear whether the pathogen was introduced along with BP virus during the exposure or was latent and manifested only due to stress induced by the BP exposure study. Compared to reovirus in *P. monodon* and *P. japonicus*, reovirus in *P. vannamei* is smaller and occurred in non-paracrystalline arrays. It occurred as unordered aggregates in the cell cytoplasm with paraspherical and non-enveloped virions ~50 nm diameter (Krol et al., 1990).

4.2. Reovirus in *P. elegans*

A reo-like virus was reported infecting the B cells but not R and F cells of the hepatopancreas of *P. elegans* collected from the Mediterranean Sea near Piran (Vogt, 1992), named *Palaemon* B-cell reo-like virus (PBRV). PBRV has similar size (52–55 nm in diameter), morphology, and localization to other reoviruses. Interestingly, the virus was observed in 2 out of 5 wild shrimp inspected and the pathogenicity of it remains unexplored.

4.3. Reovirus in *C. quadricarinatus*

The first reported reovirus in crayfish was detected from *C. quadricarinatus* in Australia by Edgerton et al. (2000). Chronic mortalities in *C. quadricarinatus* in the study were associated with a presumptive reovirus in the hepatopancreas and a putative parvovirus in the gills. The virion (35–40 nm in diameter) in this study was smaller than most reoviruses and similar to the size of reovirus cores. The authors suggested that the observed virions could be immature reovirus without double shell capsid (Edgerton et al., 2000). Another study also detected reovirus infection in the hepatopancreas of *C. quadricarinatus*, with ~55 nm diameter icosahedral viral particles (Hayakijkosol and Owens, 2011). Reovirus infected crayfish showed lethargy, poor appetite, a weakened tail-flip response, and reddish appendages and

mouthparts. Juvenile *C. quadricarinatus* showed low mortality when challenged with reovirus by injection (~20%), or by feeding (5%), which is similar to what is reported for reovirus infections in *P. monodon* and *P. japonicus* (Hayakijkosol and Owens, 2011). Partial RdRp sequence of *C. quadricarinatus* reovirus (GenBank accession: QIJ55897) had 33% amino acid identity to Beihai reo-like virus 1 (BHR1) sequence that was detected by metagenomics of mantis shrimp in China (GenBank accession: APG79086) (Hayakijkosol et al., 2021; Shi et al., 2016).

4.4. Reovirus in *M. nipponense*-MnRV (2016)

M. nipponense reovirus (MnRV), a new pathogenic agent of the freshwater prawn, was reported by Zhang et al. (2016). Diseased shrimps were a little smaller than healthy animals, but no other clinical sign was noted. Infection signs were observed only in epithelial cells of hepatopancreas. Viral particles had typical reovirus characteristics: non-enveloped, icosahedral virus with 60 nm in diameter. MnRV genome, with full length of ~23.6 kbp, revealed 10 distinctive bands with an electrophoretic pattern 5/2/3 (Zhang et al., 2016). A partial (33 residue) deduced RdRp amino acid sequence (GenBank accession: AKA43761) shared 35% identity with Kadipiro virus (Seadornaviridae) in insects (GenBank accession: AWE75154; APG79130) (Shi et al., 2016; Zhang et al., 2016; Zhang et al., 2018).

5. Conclusions and future directions

Since the first crustacean virus was described using electron microscopy (EM) in 1966, discovery of crustacean reoviruses has accelerated with the development of new discovery techniques and the increased observation of crustaceans in aquaculture. In the pre-genomics era, novel reoviruses were usually first identified in diseased animals by EM inspection of infected tissue or filtered homogenates of infected animals, such as P virus, RLV, EsRV905 and EsRV816 (Bonami, 1973; 1976; Johnson, 1977; Zhang et al., 2002). Histological examination and agarose gel electrophoresis of virus RNA have also been applied to characterize reovirus infections in crustaceans (e.g., Montanie et al., 1993; Bowers et al., 2010; Tang et al., 2011; Zhang and Bonami, 2012). For example, CsRV2 was discovered solely by agarose gel electrophoresis during a study of CsRV1 infections in *C. sapidus* collected from Brazil (Fig. 3A) (Zhao et al., 2021). Other standard molecular detection methods, such as RNA hybridizations and RT-qPCR, were then developed to rapidly detect and quantify specific viruses during an infection (Walton et al., 1999; Tang et al., 2011; Flowers et al., 2016; Zhao et al., 2020; Hayakijkosol et al., 2021). In recent years, with the development of PCR and high throughput Next Generation Sequencing (NGS), advances in metagenomics, ever-growing genome databases, and more user-friendly bioinformatics tools, vast numbers of viral genomes have

Table 2
Properties of reoviruses in shrimp, prawn and crayfish.

Host	Virion size (nm)	Viral inclusion morphology	RNA segment	RNA gel pattern	Sequence available	Genome size (nt)	Infection sites	Clinical signs	References
<i>Penaeus monodon</i>	50–70	paracrystalline	NA	NA	NA	NA	hepatopancreatic epithelial cells;	NA	Anderson et al. (1987)
<i>Penaeus japonicus</i>	61	paracrystalline	NA	NA	NA	NA	hepatopancreatic R-cells;	no burying, reddish uropods and hepatopancreas;	Tsing and Bonami (1987)
<i>Penaeus vannamei</i>	~50	non-paracrystalline	NA	NA	NA	NA	midgut epithelial cells;	NA	Krol et al. (1990)
<i>Palaeomonetes elegans</i>	52–55	NA	NA	NA	NA	NA	hepatopancreatic R and F cells;	NA	Vogt (1992)
<i>Cherax quadricarinatus</i>	~55	NA	NA	NA	RdRp	NA	hepatopancreatic B cells;	lethargy, anorexia, weak tail-flip, reddish appendages and mouthparts;	Edgerton et al. (2000)
<i>Macrobrachium nipponense</i>	~60	NA	10	5/3/2	RdRp	~23,600	hepatopancreatic epithelial cells;	small size of diseased shrimps;	Zhang et al. (2016)

Note: "NA" refers as no data available.

been identified and characterized, such as crustacean reoviruses-WLR1, BHR1 and BHR2 (Shi et al., 2016). Metagenomics and bioinformatics will have an increasing effect on identification and characterization of new viruses, which will certainly soon expand reovirus diversity in a wider range of crustaceans. These approaches are not a substitute for the essential tools of histology, EM, and infection studies to discover and characterize the biology of new viruses. So, rather than being replacement for these skill sets (knowledge base), genomics will only increase the need for resources and training to continue these crucial biological studies.

The development of virus genomics and metagenomics has provided a universal and quantifiable basis for virus classification. Traditionally, virus classifications have been based on properties such as virion morphology, genome organization, serology, host range, replication and transmission mechanism, and pathogenicity (Bao et al., 2008). Today, almost all new viral genomes are either sequenced when they are first discovered, or even have been assembled from metagenomic datasets which have scant biological data such as viral characteristics, host range and pathogenicity. A recent publication makes the case for integrating such assembled virus genomes into the ICTV classification scheme even in the absence of other data (Simmonds et al., 2017).

Virus classification based on phylogenetic analysis of genome sequences has been used increasingly in recent years (Gorbalenya and Lauber, 2017). In this minireview, a phylogenetic analysis of different *Reoviridae* genera was conducted to give a hypothetical classification of the diverse brachyuran crustacean reoviruses. Three clades of brachyuran crustacean reoviruses are evident in the RdRp amino acid-based phylogenetic tree, with new species added to two previously suggested genera -*Cardoreovirus* (Zhang et al., 2004) and *Crabreovirus* (Deng et al., 2012), and a new genus designated as *Crustareovirus* (Fig. 4). Based on the genome organization pattern and other partial sequence comparisons, P virus and W2 appear to be members of *Crabreovirus*, and EsRV816 was added to the genus *Crustareovirus* (Fig. 5). Further studies on whole genome sequencing of more brachyuran reoviruses (P virus, W2, RC84, EsRV816, SCRv) as well as reoviruses in other crustacean hosts, and more viral sequences identified from metagenomics, will surely strengthen and extend the classification of crustacean reoviruses.

Going forward, the ease of generating genome sequence when describing a novel virus and timely sharing of the sequences in public databases will help to avoid confusion about virus identities. Furthermore, genomic data may bring surprising new insights into crustacean virus ecology and evolution. For example, genomic sequences helped reveal the global movements and evolutionary origin of White Spot Syndrome Virus (WSSV) (Marks et al., 2004; Kawato et al., 2019). The similarity of P virus to CsRV1 (a.k.a, RLV or CsRV) suggests an intriguing possibility that they are variants of a single reovirus species infecting different hosts on different continents. Although P virus was identified from *M. depurator* in the Mediterranean, the host of CsRV1 (*C. sapidus*) was introduced to the Mediterranean as an invasive species since at least 1949 (Mizzan, 1993). This would raise questions about whether the virus made one or more *trans*-Atlantic journeys, either in *C. sapidus*, which is an invasive species throughout the Mediterranean, or in an unknown crustacean that travelled from east to west. An investigation of P virus in *C. sapidus* and other crab species in the Mediterranean can address this question and help us better understand the interactions of invasive species and viral diseases in the ocean as well as marine infectious disease ecology.

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Appendix A. Supplementary material

S1. NCBI accession numbers of RdRp amino acid sequences used in phylogenetic tree. S2. Alignment of RdRp amino acid sequences for the phylogenetic tree. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jip.2021.107568>.

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