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Phylogenomic characterization of red seabream iridovirus from Florida pompano *Trachinotus carolinus* maricultured in the Caribbean Sea

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Abstract

Between 2010 and 2016, six mortality events were observed in Florida pompano (*Trachinotus carolinus*) maricultured in the Dominican Republic. Histopathological examination and conventional PCR confirmed a megalocytivirus (MCV) infection in each case. Subsequently, next-generation sequencing and phylogenomic analyses confirmed that MCV DNA was present in the infected pompano tissue samples from 2010, 2014, and 2016, and each was determined to be red seabream iridovirus (RSIV). Annotation of the RSIV genome sequences identified 121 open reading frames, and BLASTN analysis revealed the highest nucleotide sequence identity (> 99%) to a RSIV clade 1 MCV isolated from a moribund red seabream (*Pagrus major*) maricultured in Japan. These cases represent the first fully sequenced RSIV genomes detected outside of Asia and are the earliest reports of MCV infections in Florida pompano. This recent geographical expansion of RSIV warrants further attention to determine its potential economic and ecological impact.

The genus *Megalocytivirus* is one of three genera within the subfamily *Alphairidovirinae* of the family *Iridoviridae* [1]. Megalocytiviruses (MCVs) are double-stranded DNA viruses that continue to cause significant economic losses to both ornamental and food fish industries. The sole MCV species, *Infectious spleen and kidney necrosis virus*, includes three genotypes: infectious spleen and kidney necrosis virus (ISKNV), red seabream iridovirus (RSIV), and turbot reddish body iridovirus (TRBIV) [2]. Red seabream iridoviral disease (RSIVD), caused by genotypes RSIV or ISKNV, is listed as one of ten reportable fish diseases by the World Organization for Animal Health (OIE) [3]. In this study, we provide the first complete RSIV genome sequences detected outside of Asia, expanding the geographic range of RSIV into the Caribbean Sea and in a novel fish host, Florida pompano (*Trachinotus carolinus*).

In 2010, 2013, 2014, and 2016, a total of six mortality events occurred in Florida pompano maricultured in the Dominican Republic (Table 1). For each of the 2010, 2014a, and 2016 events, DNA was extracted from frozen spleen or kidney tissue samples using a DNeasy Blood and Tissue Kit (QIAGEN) following the manufacturer's instructions. For the 2013, 2014b, and 2014c events, DNA was extracted from formalin-fixed paraffin-embedded tissue samples (liver, kidney, spleen, heart, intestine, eye) following a previously described protocol [2]. Histopathologic examination performed in four of the mortality events revealed cytomegalic cells with basophilic, granular, intracytoplasmic inclusions in one or more organs (liver, kidney, spleen, heart, intestine, eye), compatible with a MCV infection (Table 1). Tissue samples from all six pompano tested positive for MCV, using two specific conventional PCR assays (primers MCV-F/R and primers RSIV1-F/R) [2, 4] (Table 1). Amplified products of the expected sizes were purified using a QIAquick PCR Purification Kit (QIAGEN) and sequenced in both directions by Sanger sequencing using the aforementioned primers. BLASTN searches of the sequences revealed

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Table 1 Summary of histologic and molecular diagnostic results for Florida pompano cases from 2010 to 2016

Sample ID	Collection year	Sample type	Histology results	PCR results			Genome size (bp)
				RSIV1-F/R	MCV-F/R	Megalocytivirus clade	
PIV2010	2010	FT	N/D	+	+	RSIV clade 1	112,321
PIV2013	2013	FFPE	+	+	+	RSIV clade 1	N/D
PIV2014a	2014	FT, FFPE	+	+, +	+, +	RSIV clade 1	112,377
PIV2014b	2014	FFPE	+	+	+	RSIV clade 1	N/D
PIV2014c	2014	FFPE	+	+	+	RSIV clade 1	N/D
PIV2016	2016	FT	N/D	+	+	RSIV clade 1	112,052

FFPE, formalin-fixed paraffin-embedded tissues; FT, frozen tissue; N/D, not done

the highest nucleotide sequence identity (> 99%) to a RSIV genomic sequence (GenBank accession no. AB104413; Table 1) determined from moribund red seabream (*Pagrus major*) in Japan [5]. Hereafter, the MCVs detected in Florida pompano will be referred to as pompano iridovirus (PIV).

DNA libraries were built using a NEBNext Ultra II DNA Kit for the PIV2010 DNA sample and an Illumina TruSeq DNA PCR-Free Kit for the PIV2014a and PIV2016 DNA samples (Table S1). All three DNA libraries were sequenced using a v3 chemistry 600-cycle Kit on a MiSeq sequencer (Illumina). *De novo* assemblies of the paired-end reads were performed in SPAdes 3.10.1 [6] using default settings. The integrity of the complete genome sequences were verified by mapping the reads using Bowtie 2 [7] and inspecting the alignments in Tablet 1.17.08.17 [8]. Genomes PIV2010, PIV2014a, and PIV2016 were 112,321, 112,377 and 112,052 bp in size with an average coverage of 1371, 136, and 43 reads/nucleotide, respectively.

Open reading frames (ORFs) for PIV2010 (GenBank accession no. MK098185), PIV2014a (GenBank accession

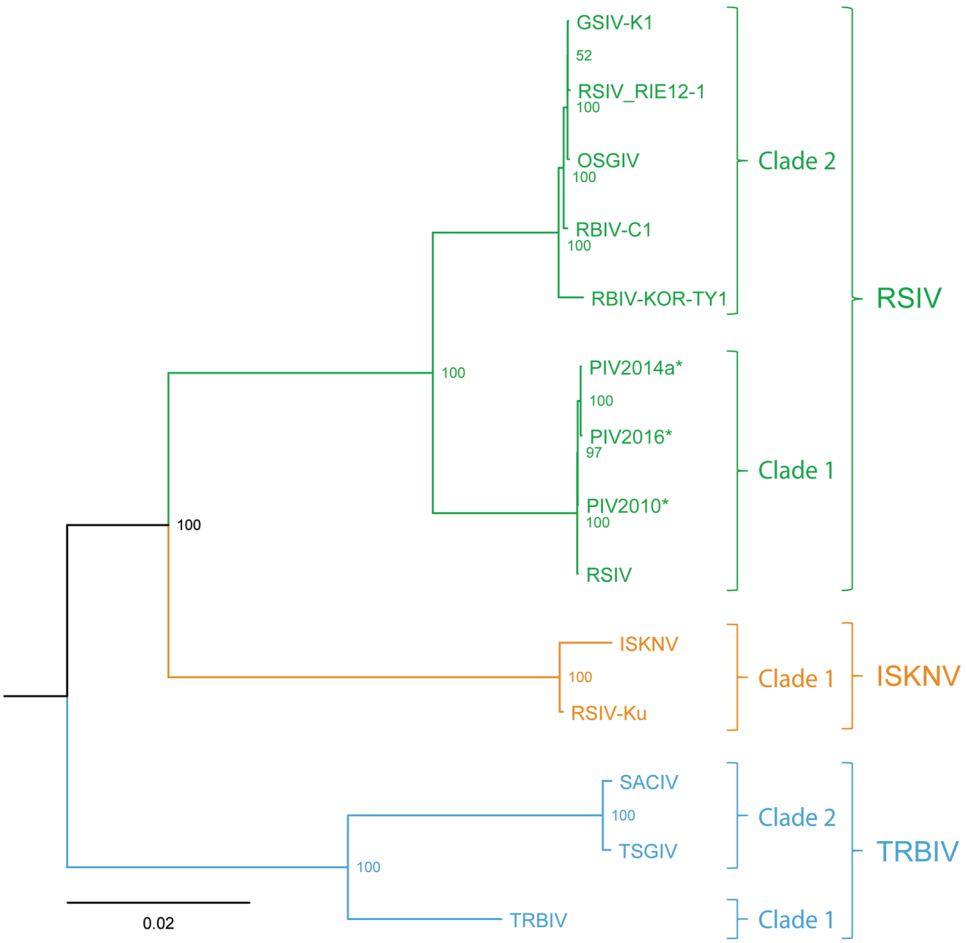

Fig. 1 Maximum-likelihood phylogram illustrating the relationship of PIV2010, PIV2014a, and PIV2016 (indicated with asterisks) to 11 other megalocytiviruses based on their aligned genome sequences. Bootstrap values are given at each node, and the branch lengths represent the number of inferred substitutions as indicated by the scale. Refer to Table S1 for virus abbreviations

no. MK098186), and PIV2016 (GenBank accession no. MK098187) were predicted using the Genome Annotation Transfer Utility (GATU) with RSIV strain Ehime-1 (GenBank accession no. AB104413) as the reference genome [9]. A total of 121 ORFs were annotated for the three genomes, and the gene functions were predicted based on BLASTP searches against the National Center for Biotechnology Information (NCBI) GenBank non-redundant protein sequence database and NCBI conserved domains database.

A phylogenomic analysis was performed to determine the relationship of the PIV (PIV2010, PIV2014a, and PIV2016) genomic sequences to 11 other previously sequenced MCVs. The 14 MCV genomes were aligned in Mauve 2.4.0 [10], and the resulting locally collinear blocks were concatenated using Geneious R10 [11]. A maximum-likelihood phylogenetic analysis was conducted using IQ-TREE 1.5.6 with the Bayesian information criterion option to determine the best model fit and 1000 bootstraps to determine clade support [12]. The analysis generated a well-resolved and supported phylogeny with all three PIV genomes grouping within RSIV clade 1 (Fig. 1).

The first well-characterized case of MCV disease, caused by a RSIV clade 1 MCV, occurred in red seabream cultured in Shikoku Island, Japan, in 1990 [5]. Since that time, there have been numerous reported cases of RSIV infection in marine fish species from China, Japan, Singapore, South Korea, Taiwan, and Thailand [13–16]. RSIV infection is currently considered reportable by the OIE, and a vaccine is commercially available for use in Japan [17]. Our study represents the earliest detection of a RSIV clade 1 MCV outside of Asia. Although it was beyond the scope of the current study to determine the role of PIV in the Florida pompano mortality events in the Dominican Republic reported here, our cases bear a striking resemblance to a previous report of RSIV clade 1 infection in Florida pompano associated with a mortality event in a Central American mariculture facility [18]. Florida pompano are an economically important commercial and sport fish for the United States, where the price per pound is high due to their limited supply [19]. Future studies are needed to determine the role of PIV in disease and any negative economic impact it has on the mariculture of Florida pompano in the Caribbean Sea.

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Compliance with ethical standards

Conflict of interest All of the authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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