Title: Identification of the first endogenous *Ophiovirus* sequence.

Authors: Soledad Marsile-Medun[1,2] and Robert James Gifford[1]

Affiliations:

1. MRC-University of Glasgow Centre for Virus Research, 464 Bearsden Road, Glasgow, UK
2. Agrocampus Ouest, 65 Rue de Saint-Brieuc, 35000 Rennes, France

Corresponding author: Robert J. Gifford, MRC-University of Glasgow Centre for Virus Research, Glasgow, UK. E-mail: robert.gifford@glasgow.ac.uk

Abstract

Endogenous viral elements (EVEs) are sequences in eukaryotic genomes that are derived from the ancestral integration of viral sequences into germline cells. Ophioviruses (family *Ophioviridae*) are a recently established family of viruses that infects plants. In this report, we describe the first example of an EVE derived from an ophiovirus, in the genome of eelgrass (*Zostera marina*). These findings extend the host range of ophioviruses to include seagrasses of the family *Zosteraceae*.

Introduction

Ophioviruses (family *Ophioviridae*) are a recently established family of viruses that infects plants, causing economically important diseases [1, 2]. Only one genus (*Ophiovirus*) is currently recognized, containing seven species (**Table 1**). All ophioviruses are characterized by non-enveloped nucleocapsids that have helical symmetry and are highly filamentous. The negative-stranded RNA linear genome contains 3 or 4 segments coding for up to seven proteins. The first segment contains two ORFs, one encoding a 22–25K protein, and a second encoding the viral RNA polymerase. The second segment encodes the cell-to-cell movement protein (MP), while the coat protein (CP) is encoded by the third. A fourth segment has been reported in ophioviruses infecting lettuce (*Lactuca sativa*), which encodes putative proteins of unknown function [1].

Endogenous viral elements (EVEs) are sequences in eukaryotic genomes that are derived from the ancestral integration of viral sequences into germline cells [3]. EVEs can provide unique retrospective information about the long-term coevolutionary history of viruses and their hosts [4, 5]. Here, we describe the first

example of an EVE derived from an ophiovirus, in the genome of eelgrass (*Zostera marina*).

Results

We screened genome assemblies of 142 plant species (**Table S1**) for sequences related to ophiovirus proteins. We identified only one statistically significant match, in the recently published genome assembly of eelgrass (*Zostera marina*) [6]. This sequence - hereafter referred to as *Zostera marina* endogenous ophioviral element (OphVe-ZosMar) spanned 567 nucleotides. When virtually translated, this sequence shared ~34-38% amino acid (aa) identity with the coat protein (CP) of known ophioviuses (**Figure 1**). The putative protein coding sequence of OphVe-ZosMar produced numerous, highly significant hits to ophioviruses when used to search GenBank, and no hits to sequences derived from other species.

The OphVe-ZosMar element was identified in a large scaffold (accession # LFYR01000112.1: positions 103766-104332), and was flanked on either side by DNA sequences that exhibited no statistically significant similarity to any other viral or plant sequences in GenBank. Furthermore, neither the OphVe-ZosMar element nor it's flanking sequences could be identified in the published genome assembly of *Zoster muelleri* [7], a related seagrass species. Assuming that the OphVe-ZosMar element is genuinely incorporated into the *Z.marina* genome (i.e. it does not reflect an artifact introduced through contamination), and is genuinely absent from the *Z.muelleri* genome, this would imply that the germline integration event that created OphVe-ZosMar occurred after these species diverged an estimated ~10-20 million years ago (MYA) [8, 9].

We used maximum likelihood to infer the phylogenetic relationships between known ophioviruses and the OphVe-ZosMar. As shown in **Figure 2**, the phylogeny discloses two well-supported subgroups within the *Ophioviridae*, one that contains the ophioviruses infecting citrus and blueberry (**group 1**), and another containing all other ophioviruses (**group 2**). In midpoint rooted trees, the OphVe-ZosMar element groups outside both of these clades. However, the branch length separating OphVe-ZosMar from these two groups of exogenous ophioviruses was not much greater than that separating the two groups from one another.

Discussion

Progress in whole genome sequencing has led to the identification of numerous, diverse EVE sequences in the eukaryotic species. Many of these EVEs are clearly derived from well-recognized virus families, whereas others are only

distantly related to contemporary viruses. Furthermore, although EVEs derived from a diverse range of virus families have been, there are still many virus families for which no EVEs have been identified.

In this paper we describe the identification of *Zostera marina* endogenous ophioviral element (OphVe-ZosMar) - the first reported example of an EVE derived from an ophiovirus. This sequence, which was derived from the segment of the ophiovirus genome that codes for the viral coat protein (CP), was identified in the recently sequenced genome of eelgrass (*Zostera marina*) [6]. The DNA sequences flanking OphVe-ZosMar did not disclose significant similarity to genome sequences identified in other plants, and were also found to be moderately repetitive. For this reason, we could not completely rule out the unlikely possibility that certain the presence of OphVe-ZosMar in a large contig reflected an artifact associated with contamination and/or misassembly during the production of the *Z. marina* genome. Future studies of seagrass genomes should allow the presence of the OphVe-ZosMar element in *Z. marina* to be confirmed. Confirmation of that the OphVe-ZosMar element occurs in eelgrass would enable further investigations, in particular, it should allow the age of the element to be estimated, providing some insight into the timeline of ophiovirus evolution, about which nothing is currently known. In addition, since the OphVe-ZosMar element appears to encode an intact (or nearly intact) CP protein, the possibility of conducting functional studies may also exist.

Seagrasses are one of several groups of angiosperms (flowering plants) that, having evolved on land, subsequently colonised the marine environment [10]. Terrestrial plants are thought to have originated during the Silurian period (~450 MYA), but it was not until ~130 million years ago that angiosperms evolved and invaded marine environments. This change might be expected to have led to marine angiosperms being exposed to a distinct set of viruses as compared to their terrestrial ancestors. The 'fossil record' of EVEs in marine angiosperm genomes can potentially provide some insight into this macroevolutionary shift, and its impact on contemporary plant and virus species.

Methods

Genome screening and sequence analysis

Plant genome assemblies (**Table S1**) were downloaded from NCBI (www.ncbi.nlm.nih.gov/genome/). Screening was performed using the database-integrated genome-screening tool (available from http://giffordlabcvr.github.io/DIGS-tool/). ORFs were inferred by manual comparison of putative peptide sequences to those of closely related exogenous parvoviruses in the alignment editing software

Se-Al [11]. The putative peptide sequences of *Ophiovirus*-related EVEs were aligned those of representative ophioviruses using MUSCLE [12] and PAL2NAL [13]. Phylogenies were reconstructed from this alignment, using maximum likelihood as implemented in RaxML [14], and the VT protein substitution model as selected using ProTest [15].

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Table 1. *Ophiovirus* reference sequences

Virus name	Abbreviation	Host	Accession
Citrus psorosis virus	CPsV	Citrus	AM159538
Citrus ringspot virus	CRsV	Citrus	AF060855
Blueberry mosaic associated virus	BlMaV	Blueberry	KJ849207
Mirafiore lettuce big-vein virus	MLBvV	Lettuce	GU139125
Lettuce big-vein associated virus	LBVaV	Lettuce	FJ864680
Tulip mild mottle mosaic virus	TMmV	Tulip	AY542958
Freesia sneak virus	FSnV	Freesia	KC771894

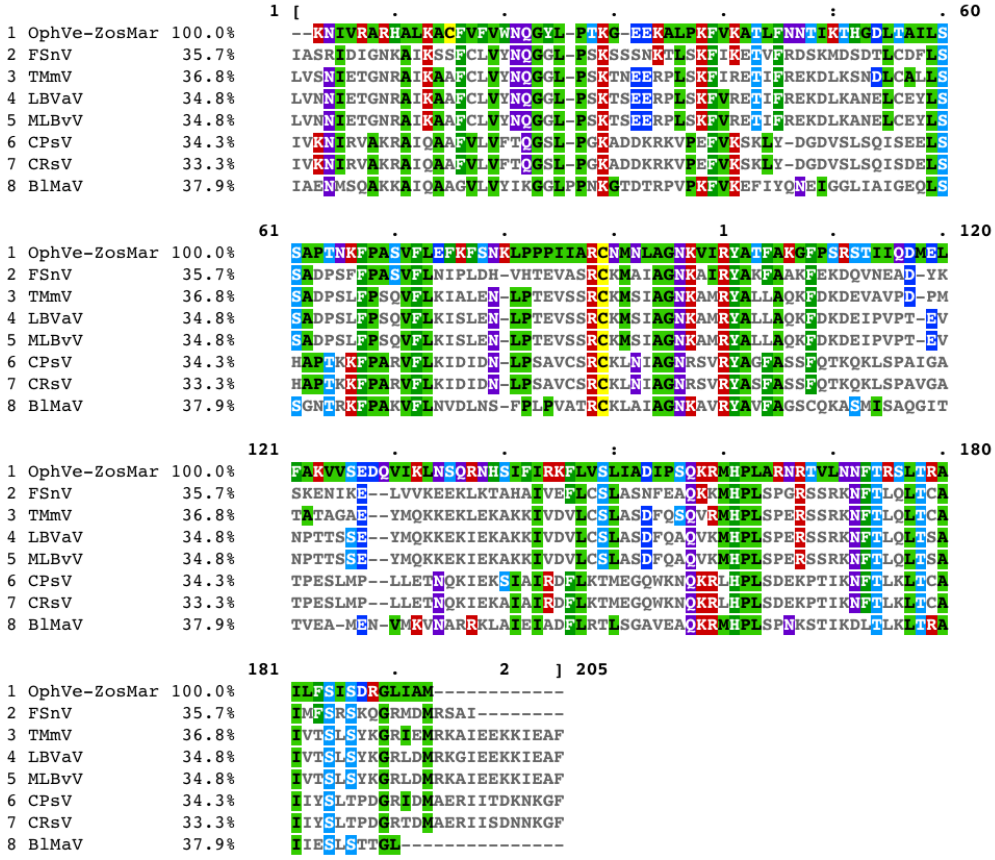

Figure 1. Alignment of ophiovirus coat protein (CP) sequences with the predicted polypeptide sequence encoded by *Zostera marina* endogenous ophiovirus (OphVe-ZosMar. Identities are shown relative to OphVe-ZosMar, and are normalised by aligned length. Residues are coloured to highlight identity and amino acid properties. Virus names are abbreviated as shown in **Table 1**.

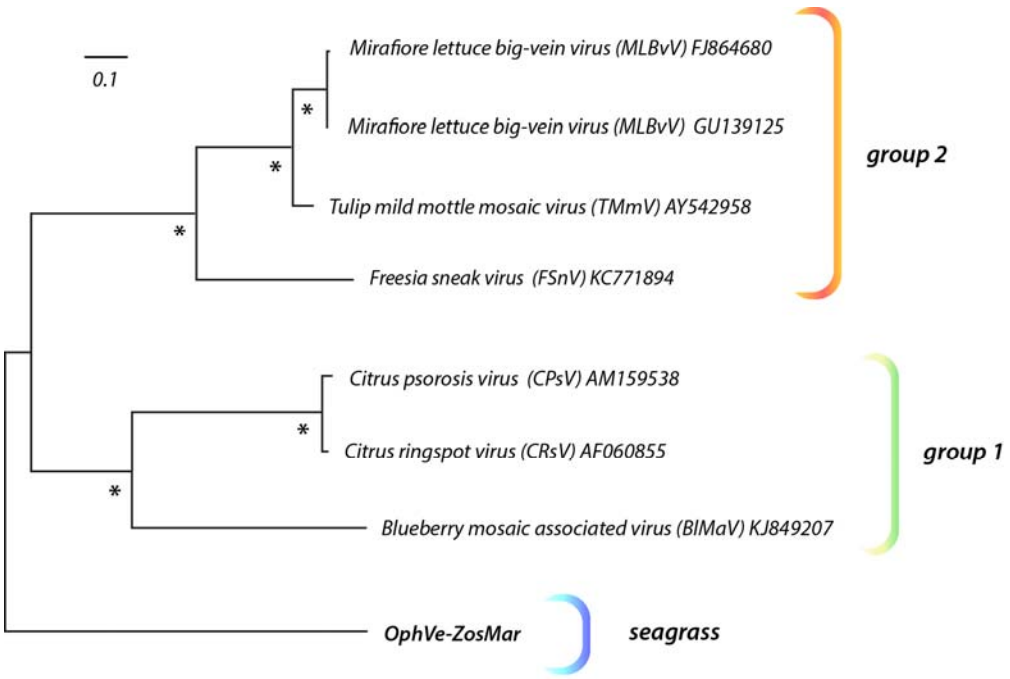

Figure 2. Bootstrapped maximum likelihood phylogeny showing the inferred evolutionary relationships between *Zostera marina* endogenous ophioviral element (OphVe-ZosMar) and representative ophioviruses. The tree was inferred from the polypeptide alignment shown in **Figure 1.** Asterisks indicate nodes with >95% bootstrap support. The scale bar shows phylogenetic distance in substitutions per site. Accession numbers of ophiovirus taxa shown here are given in **Table 1**.