Limited potexvirus diversity in seagrasses

1.1 Author names

Shen Jean Lim[1*] (https://orcid.org/0000-0003-4578-5318)

Karyna Rosario[1] (https://orcid.org/0000-0001-9847-4113)

Meredith E. Kernbach[1] (https://orcid.org/0000-0001-8161-057X)

Anthony J. Gross[1] (https://orcid.org/0000-0001-6392-5121)

Bradley T. Furman[2] (https://orcid.org/0000-0002-9284-8388)

Mya Breitbart[1] (https://orcid.org/0000-0003-3210-2899)

1.2 Affiliation(s)

[1] College of Marine Science, University of South Florida, St Petersburg, FL, USA

[2] Florida Fish and Wildlife Conservation Commission, Florida Fish and Wildlife Research Institute, Saint Petersburg, FL, USA

1.3 Corresponding author and email address

Shen Jean Lim (jeanlim@usf.edu)

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1.5 Repositories:

All sequence data have been deposited into NCBI GenBank under the accession numbers OR827689-OR827705, OR854648, OR863396, OR879052-OR879056, and PP430548-PP430571.

2. Abstract

Turtlegrass virus X, which infects the seagrass *Thalassia testudinum*, is the only potexvirus known to infect marine flowering plants. We investigated potexvirus distribution in seagrasses using a degenerate reverse transcription polymerase chain reaction (RT-PCR) assay originally designed to capture potexvirus diversity in terrestrial plants. The assay, which implements Potex-5 and Potex-2RC primers, successfully amplified a 584 nt RNA-dependent RNA polymerase (RdRp) fragment from TVX-infected seagrasses. Following validation, we screened 74 opportunistically collected, apparently healthy seagrass samples for potexviruses using this RT-PCR assay. The survey examined the host species *T. testudinum*, *Halodule wrightii*, *Halophila stipulacea*, *Syringodium filiforme*, *Ruppia maritima*, and *Zostera marina*. Potexvirus PCR products were successfully generated only from *T. testudinum* samples and phylogenetic analysis of sequenced PCR products revealed five distinct TVX sequence variants. Although the RT-PCR assay revealed limited potexvirus diversity in seagrasses, the expanded geographic distribution of TVX shown here emphasizes the importance of future studies to investigate *T. testudinum* populations across its native range and understand how the observed fine-scale genetic diversity affects host-virus interactions.

3. Impact statement

Potexviruses are widespread in terrestrial plants; however, the recent discovery of TVX in the seagrass *Thalassia testudinum* extends their host range to marine flowering plants. Here we use existing Potex-5 and Potex-2RC degenerate primers to explore potexvirus infections in several seagrass species. TVX sequence variants were detected in *T. testudinum* collected from the eastern Gulf of Mexico, uncovering previously unknown genetic diversity of this poorly understood virus.

4. Data summary

All sequence data are available in NCBI GenBank under the accession numbers OR827692-OR827705, OR854648, OR863396, OR879052-OR879056, and PP430548-PP430571. The authors confirm all supporting data, code and protocols have been provided within the article or through supplementary data files.

5. Introduction

Potexviruses are positive-sense single-stranded RNA (ssRNA) viruses from the family *Alphaflexiviridae* that infect many agronomically important flowering plants (angiosperms), including potato, tobacco, and tomato (1). The type member of this genus is *Potato virus X* (PVX), from which the genus name *Potexvirus* is derived (2). Potexviruses have a broad

55 geographic range that reflects the distribution of their hosts and the prevalence of global
56 commerce (2, 3). Although each potexvirus species has a limited natural host range, some
57 species can infect a wide range of host plants in experimental settings (1). Potexviruses are
58 transmitted mechanically through contaminated agricultural equipment or plant-to-plant
59 contact (1-3). Transmission via seed and aphid vectors and non-specific transmission through
60 chewing insects has been reported but is less common (1-3). Most potexviruses cause
61 persistent, asymptomatic infections or mild mosaic disease, while some cause dwarf,
62 necrotic, or ringspot symptoms in their natural hosts (1, 2). Notably, PVX can cause severe
63 symptoms when its plant host is co-infected with potyviruses (4-6).

64

65 In addition to infecting terrestrial plants, potexviruses are also capable of infecting
66 seagrasses, the only marine angiosperms. A novel potexvirus species, *Turtlegrass virus X*
67 (TVX), was discovered in apparently healthy *Thalassia testudinum* seagrass samples collected
68 from Terra Ceia Aquatic Preserve, Tampa Bay, Florida, USA (7). The identification of TVX
69 indicates that an important aspect of potexvirus diversity has been overlooked by focusing
70 only on terrestrial plants. Degenerate primers used in reverse transcriptase polymerase
71 chain reaction (RT-PCR) assays, such as Potex-5 and Potex-2RC (8), have proven useful in
72 detecting diverse potexviruses, but have not been tested in aquatic habitats. This study
73 applied the Potex-5 and Potex-2RC primer pair in a RT-PCR assay to explore potexvirus
74 diversity in samples from six seagrass host species.

75 6. Methods

76 ***Primer validation*.** Potex-5/Potex-2RC were tested on cDNA samples each synthesized from 1
77 µg RNA extracted from *Portulaca* sp. infected with *Alternanthera mosaic virus* (AltMV) and
78 *Opuntia* sp. with a mixed infection of potexviruses, as well as cDNA pooled from *T.*
79 *testudinum* samples known to contain TVX, based on the TVX-specific RT-PCR assay
80 described by Van Bogaert *et al.* 2019 (7). The PCR reaction contained 0.48 µM of each
81 primer, 2 µL cDNA template, 1 µL GC enhancer, and 1X AmpliTaq Gold™ 360 Master Mix
82 (Applied Biosystems™, Waltham, MA, USA) in a 25 µL reaction volume. PCR was performed
83 under the following conditions: initial denaturation at 95°C for 10 minutes, 40 cycles of
84 denaturation at 95°C for 30 seconds, annealing at 51.5°C (as published in van der Vlugt and
85 Berendsen (8)) for 30 seconds, extension at 72°C for 1 minute, followed by elongation at
86 72°C for 10 minutes and cooling at 11°C. The PCR product was visualized following gel
87 electrophoresis on a 1% (wt/vol) agarose gel stained with ethidium bromide. All PCR
88 reactions, except for the no template control, yielded visible bands. All PCR products were
89 purified using the Zymoclean Gel DNA Recovery Kit (Irvine, CA, USA), quantified using the
90 Qubit™ DNA high sensitivity (HS) assay (Invitrogen™, Waltham, MA, USA), and Sanger
91 sequenced bidirectionally by Eurofins Genomics (Louisville, KY, USA).

92 ***RT-PCR***. Total RNA extraction was performed on 30-100 mg of leaves from multiple shoots
93 pooled by seagrass species and collection site (**Table S1**) using Zymo Research's (ZR) Quick-
94 RNA™ Plant Miniprep kit. Each pooled seagrass sample was homogenized in a BashingBead
95 Lysis Tube containing 2 mm ceramic beads and 800 µL RNA lysis buffer (provided in the kit)
96 for 5 minutes at maximum speed using a Fisherbrand™ Bead Mill 4 Homogenizer (Fisher
97 Scientific, Waltham, MA, USA). Tissue homogenates were centrifuged at maximum speed
98 (21,130 *g*) for 1 minute and RNA was extracted from the total volume (~800 µL) of the
99 supernatant. To ensure successful RNA extraction, after each round of extraction, a random
100 subset of RNA samples was quantified using the Qubit™ RNA high sensitivity (HS) assay
101 (Invitrogen™). From each sample, cDNA was synthesized from 8 µL RNA using the
102 SuperScript™ IV First-Strand Synthesis System (Invitrogen™) and following manufacturer's
103 instructions for random hexamers. PCR amplification was performed on each cDNA sample,
104 followed by gel electrophoresis, PCR product purification, DNA quantification, and Sanger
105 sequencing, using the methods described above.

106

107 ***Amplicon sequence analysis*.** Amplicon sequences were first compared with sequences from
108 NCBI's nucleotide (nt) and non-redundant protein (nr) collections (9) and the TVX genome
109 (7) using the megablast and/or blastx programs on NCBI's BLAST® server (10). Sequence
110 reads from each sample were then mapped to the Potex-5/Potex-2RC amplicon region (with
111 forward and reverse primer sequences removed) in the TVX genome (7) using the "Map
112 Sanger Reads to Reference" function implemented in Unipro UGENE v48.1 (11), with a
113 trimming quality threshold of 20 and mapping minimum similarity of 70%.
114 Electropherograms of mapped reads were manually inspected to remove primer sequences
115 and low-quality bases and to resolve ambiguous bases.

116

117 Sequences from 42 Potex-5/Potex-2RC PCR products amplified from seagrass and terrestrial
118 plant samples described above were aligned with corresponding amplicon regions (excluding
119 primer sequences) extracted from the reference genomes of TVX (NC_040644) (7), *Bamboo*
120 *mosaic virus* (NC_001642) (12), *Foxtail mosaic virus* (NC_001483) (13), AltMV (OR607766),
121 PVX (NC_011620) (14), and *Lolium latent virus* (NC_010434) (15) retrieved from the NCBI
122 Virus database (16). Multiple sequence alignment was performed using the L-INS-I and --
123 adjustdirectionaccurately options in MAFFT v7.5.08 (17), then trimmed to 514 nt to remove
124 leading and trailing gaps. Pairwise sequence identities were calculated from the trimmed
125 alignment using Clustal Omega v1.2.3 (18) with the --distmat-out, --percent-id, and --full
126 options. The pairwise sequence identity matrix was clustered, based on Euclidean distances,
127 using the hierarchical clustering with complete linkage method implemented in the
128 heatmap.2 function of the gplots R package (https://github.com/talgalili/gplots).

For phylogenetic analysis, Molecular Evolutionary Genetics Analysis (MEGA) 11 (19) identified the best model for the trimmed alignment to be the Hasegawa-Kishino-Yano model (20) with a discrete Gamma distribution (HKY+G). Using this substitution model, a maximum likelihood tree with 1,000 bootstrap replicates was constructed from the alignment using MEGA11 (19). The tree was condensed using MEGA11 (19) to eliminate interior branches with <50% bootstrap support. The final tree was visualized and annotated using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/Figtree/).

7. Results

The Potex-5/Potex-2RC primer pair successfully amplified TVX from *T. testudinum* samples collected from Terra Ceia Aquatic Preserve, AltMV infecting *Portulaca* sp., and an uncharacterized potexvirus infecting *Opuntia* sp. (8). A RT-PCR assay using Potex-5/Potex-2RC was subsequently performed on seagrass leaves opportunistically collected from separate sampling efforts, including 1) *T. testudinum*, *H. wrightii*, *S. filiforme*, and *R. maritima* from Tampa Bay on Jan 26th or Feb 4th, 2022; 2) *T. testudinum* from the Dry Tortugas National Park, Florida, USA collected between May 16th-20th, 2022; 3) *T. testudinum* from a systematic seagrass survey at Terra Ceia Aquatic Preserve on August 1st, 2022; and 4) *T. testudinum*, *H. wrightii*, and *S. filiforme* samples collected from Tampa Bay seagrass site S3T8 (Lassing Park) on October 3rd, 2023 (**Figure 1** and **Table S1**). Seagrass samples (**Table S1**) were also received from other research groups, including *T. testudinum* from Panacea (Florida, USA), *Zostera marina* from York River (Virginia, USA), West Falmouth Harbor (Massachusetts, USA), Sitka (Alaska, USA), New Zealand, and Kalmar (Sweden), and *Halophila stipulacea* from Jobos Bay National Estuarine Research Reserve (Puerto Rico, USA). PCR products were successfully amplified and sequenced from 40 *T. testudinum* samples from Florida sites, including Terra Ceia Aquatic Preserve, Tampa Bay seagrass sites S1T5 and S3T8 (Lassing Park), Panacea located in the Florida Panhandle, and Florida Keys sites including Bush Key, Garden Key, Marquesas Key, and Key West (**Figure 1**). Aside from *T. testudinum*, no other seagrass species produced PCR products.

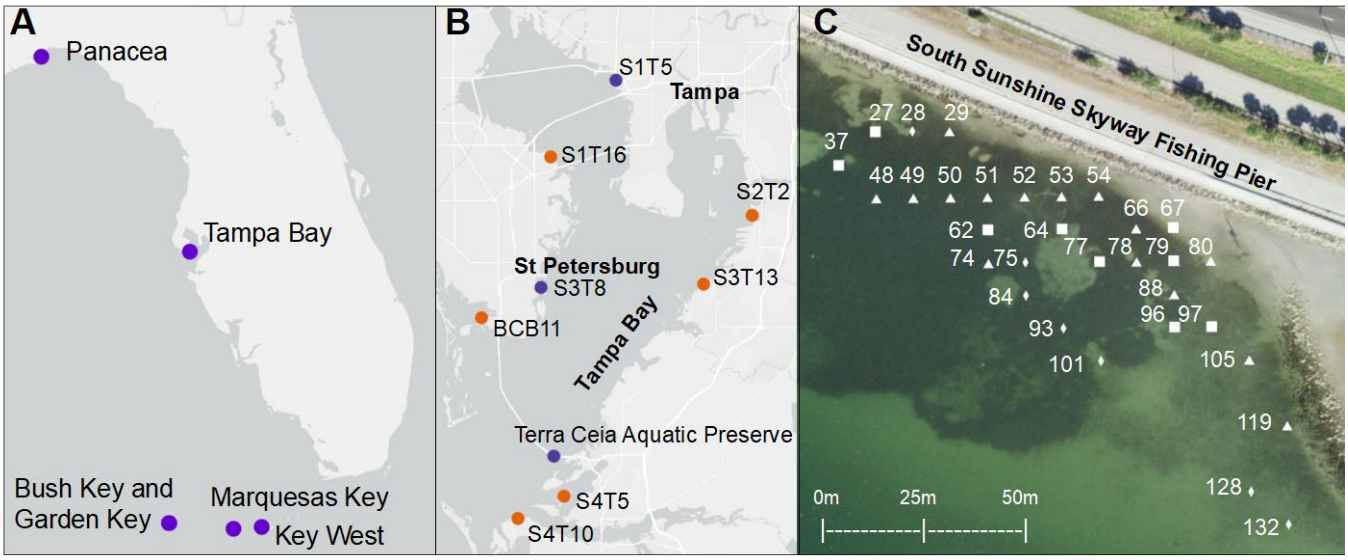


Figure 1. Map showing locations of seagrass sites in (A) Florida, (B) Tampa Bay, and (C) Terra Ceia Aquatic Preserve where samples were collected for RT-PCR. Purple markers in (A) and (B) denote sites where TVX products were amplified from *T. testudinum* with the Potex-5/Potex-2RC primers, whereas samples from sites with orange markers tested negative. Marker shapes in (C) represent potexvirus subclades found in Terra Ceia Aquatic Preserve sampling sites and correspond to **Figure 2** and **Figure 3** (negative samples not shown). Metadata of seagrass samples are described in **Table S1**.

Phylogenetic analysis placed all Potex-5/Potex-2RC amplicons from seagrasses in the same clade as the RdRp sequence fragment from the TVX genome (7) with 100% bootstrap confidence (**Figure 2**). TVX sequences from Terra Ceia Aquatic Preserve formed three subclades. Average pairwise nucleotide sequence identities were 95.8±1.4% within each subclade and 79.3±2.9% between each subclade (**Figure 3**). Two Terra Ceia Aquatic Preserve subclades, including the subclade containing the amplicon region from the TVX genome, were sister groups to each other (**Figure 2**), sharing 84.6±1% average pairwise nucleotide sequence identities (**Figure 3**). Other TVX sequences from Key West, Marquesas Key, and Panacea formed a subclade with 99.6±0.2% average pairwise nucleotide sequence identities, while sequences from Garden Key and Bush Key formed another subclade with 89.3±0.9%% average pairwise nucleotide sequence identities (**Figure 2** and **Figure 3**). Potexviruses that infect terrestrial grasses (*Foxtail mosaic virus* and *Bamboo mosaic virus*) formed sister clades to the TVX-containing clade (**Figure 2**).

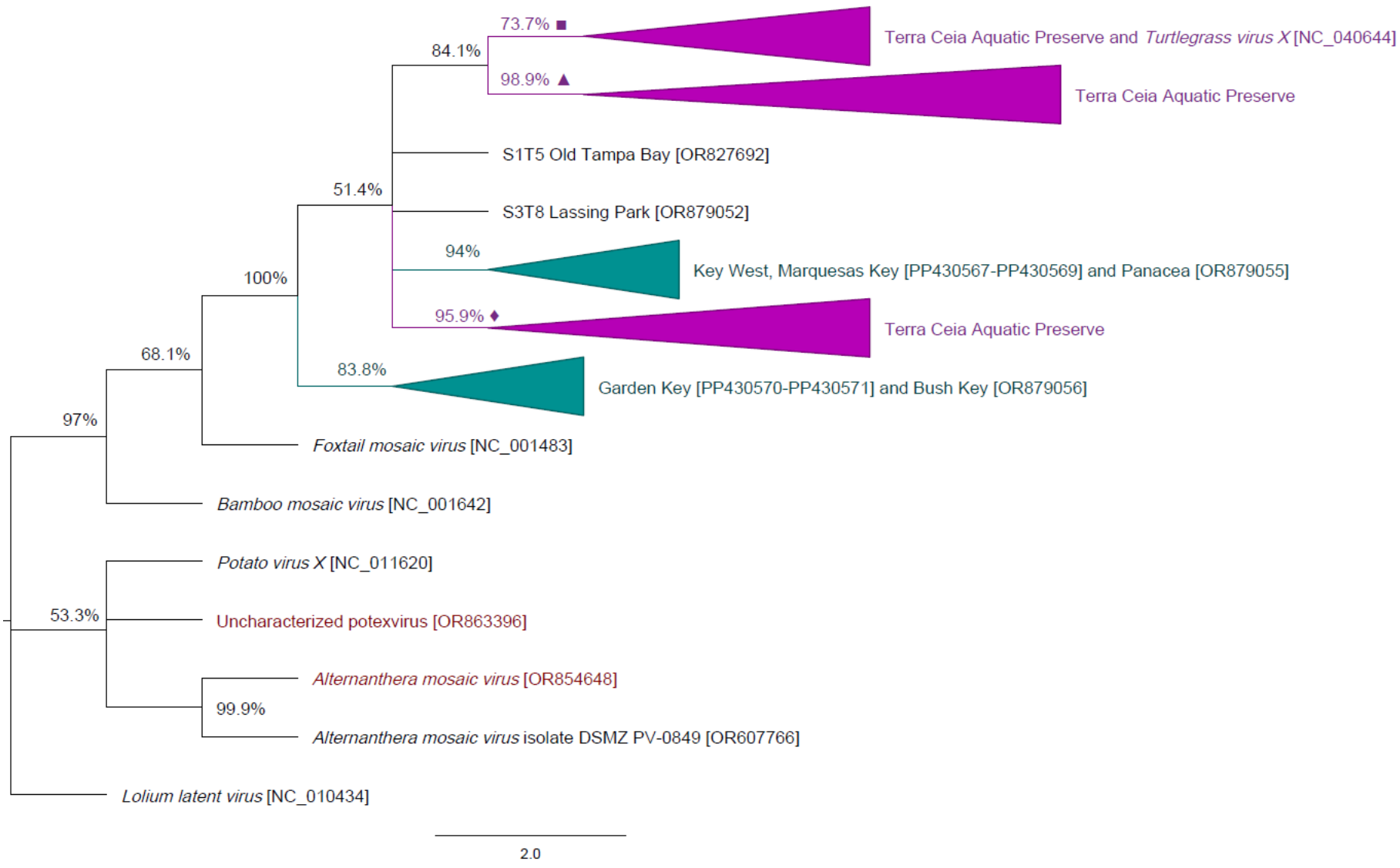
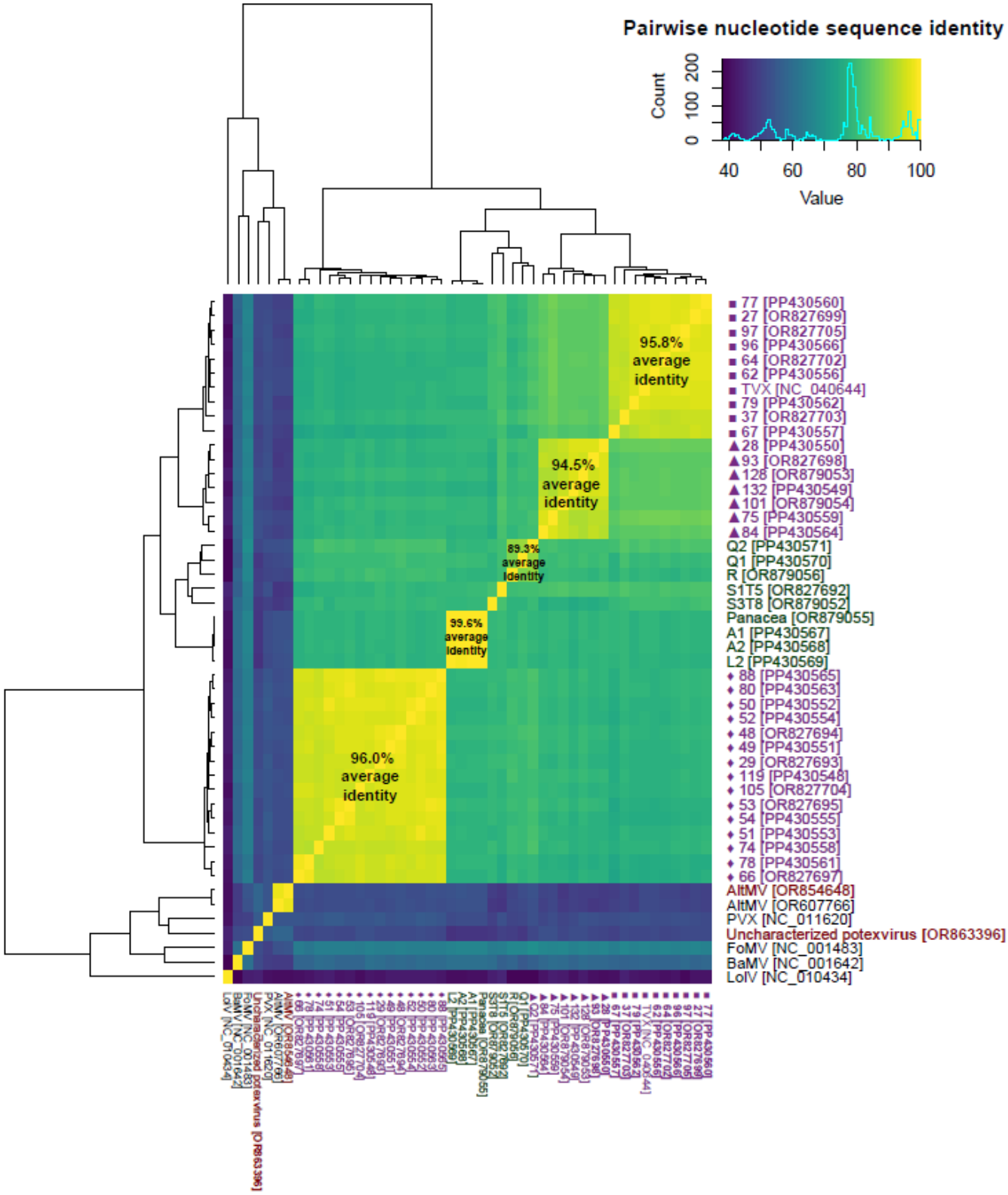

73.7% ■
84.1%
98.9% ▲
Terra Ceia Aquatic Preserve and *Turtlegrass virus X* [NC_040644]
Terra Ceia Aquatic Preserve
S1T5 Old Tampa Bay [OR827692]
51.4%
S3T8 Lassing Park [OR879052]
94%
Key West, Marquesas Key [PP430567-PP430569] and Panacea [OR879055]
100%
95.9% ♦
Terra Ceia Aquatic Preserve
68.1%
83.8%
Garden Key [PP430570-PP430571] and Bush Key [OR879056]
97%
Foxtail mosaic virus [NC_001483]
Bamboo mosaic virus [NC_001642]
Potato virus X [NC_011620]
53.3%
Uncharacterized potexvirus [OR863396]
Alternanthera mosaic virus [OR854648]
99.9%
Alternanthera mosaic virus isolate DSMZ PV-0849 [OR607766]
Lolium latent virus [NC_010434]
2.0

Figure 2. Condensed bootstrap consensus tree of TVX nucleotide sequences amplified from seagrasses (purple text), in relation to potexvirus sequences amplified from terrestrial plants (brown text) and potexvirus RdRp sequences from reference genomes (black text). The NCBI accession number for each sequence is indicated in square brackets. Accession numbers for sequences from Terra Ceia Aquatic Preserve samples are listed in **Figure 3**. The tree was constructed by MEGA11 from a 514 nt conserved region in the multiple sequence alignment. Branch lengths indicate the number of substitutions per site. Bootstrap values on tree nodes indicate the percentage of trees, based on 1,000 replicates, in which sequences within a node were clustered together. Shapes beside bootstrap values of Terra Ceia Aquatic Preserve subclades correspond to shapes in **Figure 1C**. *Lolium latent virus* was used as an outgroup.


Pairwise nucleotide sequence identity
Count
0
100
200
40
60
80
100
Value
95.8% average identity
94.5% average identity
89.3% average identity
99.6% average identity
96.0% average identity
77 [PP430560]
27 [OR827699]
97 [OR827705]
96 [PP430566]
64 [OR827702]
62 [PP430556]
TVX [NC_040644]
79 [PP430562]
37 [OR827703]
67 [PP430557]
28 [PP430550]
93 [OR827698]
128 [OR879053]
132 [PP430549]
101 [OR879054]
75 [PP430559]
84 [PP430564]
Q2 [PP430571]
Q1 [PP430570]
R [OR879056]
S1T5 [OR827692]
S3T8 [OR879052]
Panacea [OR879055]
A1 [PP430567]
A2 [PP430568]
L2 [PP430569]
88 [PP430565]
80 [PP430563]
50 [PP430552]
52 [PP430554]
48 [OR827694]
49 [PP430551]
29 [OR827693]
119 [PP430548]
105 [OR827704]
53 [OR827695]
54 [PP430555]
51 [PP430553]
74 [PP430558]
78 [PP430561]
66 [OR827697]
AltMV [OR854648]
AltMV [OR607766]
PVX [NC_011620]
Uncharacterized potexvirus [OR863396]
FoMV [NC_001483]
BaMV [NC_001642]
LolV [NC_010434]

187

188 **Figure 3.** Histogram (upper right corner), heatmap, and dendrogram (hierarchical clustering)
189 of pairwise nucleotide sequence identities between TVX sequences amplified from
190 seagrasses (purple text), potexvirus sequences amplified from terrestrial plants (brown text),
191 and potexvirus RdRp sequences from reference genomes (black text). The NCBI accession
192 number for each sequence is indicated in square brackets. Marker shapes preceding Terra
193 Ceia Aquatic Preserve seagrass sample names correspond to shapes in **Figure 1C**.
194 Abbreviations: TVX, *Turtlegrass virus X*; AltMV, *Alternanthera mosaic virus*; PVX, *Potato virus*
195 *X*; LolV, *Lolium latent virus*; FoMV, *Foxtail mosaic virus*; BaMV, *Bamboo mosaic virus*.

8. Discussion

In this study, we validated the ability of the potexvirus degenerate primers, Potex-5/Potex-2RC, published by van der Vlugt and Berendsen (8), to amplify TVX-infected seagrass samples and potexvirus-infected terrestrial plant samples. Potex-5/Potex-2RC were used in a RT-PCR survey to test six seagrass species for potexviruses. Among the seagrass species examined, potexvirus infection was only detected in *T. testudinum*, the species from which TVX was identified. Notably, the only potexviruses identified in this study were related to TVX, suggesting limited potexvirus diversity in marine angiosperms. RT-PCR analysis revealed *T. testudinum* as a natural host for TVX and confirmed TVX infection in eastern Gulf of Mexico seagrass meadows, including those in the northwest (Panacea), west-central (Tampa Bay), and southwest (Florida Keys) Florida. Three distinct TVX sequence variants were observed within a single seagrass meadow at Terra Ceia Aquatic Preserve, along with two additional TVX sequence variants in other Tampa Bay sites, Florida Keys, and Panacea.

Among all sites sampled in this study, the most extensive TVX survey was conducted within the Terra Ceia Aquatic Preserve. Systematic RT-PCR surveys at other sites will be useful in elucidating potexvirus diversity in seagrasses and in resolving potexvirus sequence variants into species or strains. The sampling scheme in this study was limited to *T. testudinum* populations along the eastern Gulf of Mexico. However, *T. testudinum* has a widespread geographic range across the Western Atlantic Ocean, with high genetic diversity that is generally partitioned into two clusters corresponding to the Gulf of Mexico or the Caribbean phylogeographic regions (21). Future potexvirus surveys covering the total distribution range of *T. testudinum*, the related species *T. hemprichii*, and other seagrass species have the potential to uncover relationships between potexvirus diversity, host diversity, and their phylogeographic distributions.

9. Author statements

9.1 Author contributions

SJL: Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Visualization, Writing - original draft

KRC: Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing.

MEK: Investigation, Writing – review & editing

AJG: Investigation, Writing - review & editing

BTF: Funding acquisition, Investigation, Writing - review & editing

MB: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - review & editing.

9.2 Conflicts of interest

The author(s) declare that there are no conflicts of interest.

9.3 Funding information

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9.4 Ethical approval

N/A

9.5 Consent for publication

N/A

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Table S1. Metadata of seagrass samples used in the RT-PCR survey. Samples successfully amplified by Potex-5/Potex-2RC primers are highlighted in bold and the NCBI accession numbers of their amplicon sequences are listed in the last column.

Sample ID	State, Country	Region	Collection Date	Site Description	Latitude	Longitude	Species	Accession
S1T5 Tt	**FL, USA**	**Upper Tampa Bay**	**4-Feb-22**	**S1T5 - Old Tampa Bay**	**27.95788**	**-82.54663**	***Thalassia testudinum***	**OR827692**
S1T5 Sf	FL, USA	Upper Tampa Bay	4-Feb-22	S1T5 - Old Tampa Bay	27.95788	-82.54663	*Syringodium filiforme*	
S1T5 Rm	FL, USA	Upper Tampa Bay	4-Feb-22	S1T5 - Old Tampa Bay	27.95788	-82.54663	*Ruppia maritima*	
S1T16 Tt	FL, USA	Upper Tampa Bay	4-Feb-22	S1T16 - Old Tampa Bay	27.88228	-82.62015	*Thalassia testudinum*	
S1T16 Sf	FL, USA	Upper Tampa Bay	4-Feb-22	S1T16 - Old Tampa Bay	27.88228	-82.62015	*Syringodium filiforme*	
S1T16 Rm	FL, USA	Upper Tampa Bay	4-Feb-22	S1T16 - Old Tampa Bay	27.88228	-82.62015	*Ruppia maritima*	
S1T16 Hw	FL, USA	Upper Tampa Bay	4-Feb-22	S2T2 - Hillsborough Bay	27.82347	-82.39305	*Halodule wrightii*	
S1T16 Rm	FL, USA	Upper Tampa Bay	4-Feb-22	S2T2 - Hillsborough Bay	27.82347	-82.39305	*Ruppia maritima*	
S3T8 Tt 2021	FL, USA	Upper Tampa Bay	26-Jan-22	S3T8 - Lassing Park	27.75327	-82.63035	*Thalassia testudinum*	
S3T8 Sf 2021	FL, USA	Upper Tampa Bay	26-Jan-22	S3T8 - Lassing Park	27.75327	-82.63035	*Syringodium filiforme*	
S3T8 Rm 2021	FL, USA	Upper Tampa Bay	26-Jan-22	S3T8 - Lassing Park	27.75327	-82.63035	*Ruppia maritima*	
S3T8 Tt 2023	**FL, USA**	**Upper Tampa Bay**	**3-Oct-23**	**S3T8 - Lassing Park**	**27.75327**	**-82.63035**	***Thalassia testudinum***	**OR879052**
S3T8 Sf 2023	FL, USA	Upper Tampa Bay	26-Jan-22	S3T8 - Lassing Park	27.75327	-82.63035	*Syringodium filiforme*	
S3T8 Rm 2023	FL, USA	Upper Tampa Bay	26-Jan-22	S3T8 - Lassing Park	27.75327	-82.63035	*Ruppia maritima*	
S3T13 Tt	FL, USA	Upper Tampa Bay	4-Feb-22	S3T13 - Apollo Beach	27.7566	-82.44758	*Thalassia testudinum*	
S3T13 Hw	FL, USA	Upper Tampa Bay	4-Feb-22	S3T13 - Apollo Beach	27.7566	-82.44758	*Halodule wrightii*	
S4T5 Tt	FL, USA	Lower Tampa Bay	26-Jan-22	S4T5 - Terra Ceia Bay	27.54417	-82.60615	*Thalassia testudinum*	
S4T5 Sf	FL, USA	Lower Tampa Bay	26-Jan-22	S4T5 - Terra Ceia Bay	27.54417	-82.60615	*Syringodium filiforme*	

S4T5 Hw	FL, USA	Lower Tampa Bay	26-Jan-22	S4T5 - Terra Ceia Bay	27.54417	-82.60615	*Halodule wrightii*	
S4T5 Rm	FL, USA	Lower Tampa Bay	26-Jan-22	S4T5 - Terra Ceia Bay	27.54417	-82.60615	*Ruppia maritima*	
S4T10 Tt	FL, USA	Lower Tampa Bay	26-Jan-22	S4T10 - Lower Tampa Bay	27.52134	-82.65712	*Thalassia testudinum*	
S4T10 Sf	FL, USA	Lower Tampa Bay	26-Jan-22	S4T10 - Lower Tampa Bay	27.52134	-82.65712	*Syringodium filiforme*	
S4T10 Hw	FL, USA	Lower Tampa Bay	26-Jan-22	S4T10 - Lower Tampa Bay	27.52134	-82.65712	*Halodule wrightii*	
S4T10 Rm	FL, USA	Lower Tampa Bay	26-Jan-22	S4T10 - Lower Tampa Bay	27.52134	-82.65712	*Ruppia maritima*	
BCB11 Tt	FL, USA	Lower Tampa Bay	26-Jan-22	BCB11 - Boca Ciega Bay	27.72243	-82.69762	*Thalassia testudinum*	
BCB11 Sw	FL, USA	Lower Tampa Bay	26-Jan-22	BCB11 - Boca Ciega Bay	27.72243	-82.69762	*Syringodium filiforme*	
BCB11 Hw	FL, USA	Lower Tampa Bay	26-Jan-22	BCB11 - Boca Ciega Bay	27.72243	-82.69762	*Halodule wrightii*	
27	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58404412**	**-82.61651603**	***Thalassia testudinum***	**OR827699**
28	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58404505**	**-82.61643497**	***Thalassia testudinum***	**PP430550**
29	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58404597**	**-82.61635392**	***Thalassia testudinum***	**OR827693**
37	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58397095**	**-82.61659599**	***Thalassia testudinum***	**OR827703**
48	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.5838997**	**-82.61651385**	***Thalassia testudinum***	**OR827694**
49	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58390062**	**-82.61643288**	***Thalassia testudinum***	**PP430551**
50	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58390163**	**-82.61635182**	***Thalassia testudinum***	**PP430552**
51	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58390255**	**-82.61627077**	***Thalassia testudinum***	**PP430553**
52	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58390347**	**-82.6161898**	***Thalassia testudinum***	**PP430554**
53	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.5839044**	**-82.61610875**	***Thalassia testudinum***	**OR827695**
54	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.5839054**	**-82.61602778**	***Thalassia testudinum***	**PP430555**

62	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.5838303**	**-82.61626977**	***Thalassia testudinum***	**PP430556**
64	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58383223**	**-82.61610774**	***Thalassia testudinum***	**OR827702**
66	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58383407**	**-82.61594564**	***Thalassia testudinum***	**OR827697**
67	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58383508**	**-82.61586467**	***Thalassia testudinum***	**PP430557**
74	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58375813**	**-82.61626868**	***Thalassia testudinum***	**PP430558**
75	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58375905**	**-82.61618771**	***Thalassia testudinum***	**PP430559**
77	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58376098**	**-82.6160256**	***Thalassia testudinum***	**PP430560**
78	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.5837619**	**-82.61594463**	***Thalassia testudinum***	**PP430561**
79	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58376283**	**-82.61586358**	***Thalassia testudinum***	**PP430562**
80	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58376383**	**-82.61578253**	***Thalassia testudinum***	**PP430563**
84	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58368689**	**-82.61618662**	***Thalassia testudinum***	**PP430564**
88	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58369066**	**-82.61586249**	***Thalassia testudinum***	**PP430565**
93	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58361564**	**-82.61610456**	***Thalassia testudinum***	**OR827698**
96	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58361849**	**-82.61586148**	***Thalassia testudinum***	**PP430566**
97	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58361941**	**-82.61578043**	***Thalassia testudinum***	**OR827705**
101	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58354439**	**-82.61602242**	***Thalassia testudinum***	**OR879054**
105	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58354817**	**-82.61569837**	***Thalassia testudinum***	**OR827704**
119	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58340475**	**-82.61561522**	***Thalassia testudinum***	**PP430548**
128	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58325941**	**-82.6156941**	***Thalassia testudinum***	**OR879053**
132	**FL, USA**	**Lower Tampa Bay**	**1-Aug-22**	**Terra Ceia Aquatic Preserve**	**27.58318816**	**-82.61561204**	***Thalassia testudinum***	**PP430549**

A1	**FL, USA**	**Dry Tortugas National Park**	**20-May-22**	**Key West**	**24.581857**	**-81.799379**	***Thalassia testudinum***	**PP430567**
A2	**FL, USA**	**Dry Tortugas National Park**	**20-May-22**	**Key West**	**24.581865**	**-81.799295**	***Thalassia testudinum***	**PP430568**
L1	FL, USA	Dry Tortugas National Park	19-May-22	Marquesas Key	24.560963	-82.125589	*Thalassia testudinum*	
L2	**FL, USA**	**Dry Tortugas National Park**	**19-May-22**	**Marquesas Key**	**24.560981**	**-82.12555**	***Thalassia testudinum***	**PP430569**
Q1	FL, USA	Dry Tortugas National Park	17-May-22	Garden Key	24.626714	-82.874075	*Thalassia testudinum*	**PP430570**
Q2	FL, USA	Dry Tortugas National Park	17-May-22	Garden Key	24.626673	-82.874162	*Thalassia testudinum*	**PP430571**
R	**FL, USA**	**Dry Tortugas National Park**	**16-May-22**	**Bush Key**	**24.626357**	**-82.868817**	***Thalassia testudinum***	**OR879056**
Y	FL, USA	Dry Tortugas National Park	18-May-22	Loggerhead Key	24.635007	-82.921341	*Thalassia testudinum*	
Panacea	**FL, USA**	**Florida Panhandle**	**10-May-23**	**Panacea**	**29.99911**	**-84.35056**	***Thalassia testudinum***	**OR879055**
MAIRIM	Puerto Rico	Jobos Bay National Estuarine Research Reserve	1-Feb-23	MAIRIM Site	Not available	Not available	*Halophila stipulacea*	
CPL	Puerto Rico	Jobos Bay National Estuarine Research Reserve	1-Feb-23	Cayo Puerca Lulu	Not available	Not available	*Halophila stipulacea*	
Z1	VA, USA	York River	2-Aug-17	York River State Park	Not available	Not available	*Zostera marina*	
Z2	MA, USA	West Falmouth Harbor	Not available	West Falmouth Harbor	Not available	Not available	*Zostera marina*	
Z3	AK, USA	Sitka	Not available	Sitka	Not available	Not available	*Zostera marina*	
Z4	New Zealand	Not available	Dec-22	New Zealand	Not available	Not available	*Zostera marina*	
Z5	Kalmar, Sweden	Not available	Not available	Kalmar	Not available	Not available	*Zostera marina*	

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