

GENOME SEQUENCING

The Complete Genome Sequences of *Halophila stipulacea* and *Syringodium filiforme*: Advancing Genomic Resources for two Tropical Caribbean Seagrasses

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Biodiversity Genomes

High-quality genomes are rapidly increasing across seagrasses. However, it remains limited for tropical seagrasses of the Hydrocharitaceae family, with most research focused on temperate seagrasses such as *Posidonia* spp. and *Zostera* spp. We present the complete genome sequences of two species of tropical Caribbean seagrasses, *Syringodium filiforme* and *Halophila stipulacea*. Illumina sequencing was performed on genetic material from wild-collected specimens. The reads were assembled using a *de novo* method followed by a finishing step. The raw and assembled data are publicly available via Genbank. These genomes will be useful for comparison studies, specifically studying adaptations linked to invasion success. Furthermore, *H. stipulacea* is the world's most invasive seagrass, colonizing new habitats in the Mediterranean and Caribbean Seas where it can transform native coastal ecosystems. This work expands on seagrass research efforts, highlighting the power of long-read assemblies to retrieve high-quality genomes.

Introduction

Seagrasses are the only marine angiosperm (flowering plants) that have secondarily adapted to the marine environment, evolving independently from three freshwater lineages within the order Alismatales. These adaptations have enabled them to root in anoxic sediments and tolerate chronic light limitation (Larkum, Orth, and Duarte 2006). Despite these remarkable traits, seagrasses are rapidly declining, with an estimated loss of one hectare of habitat every hour due to human impacts and environmental stressors (Cullen-Unsworth et al. 2018).

Globally, there are approximately 60 species of seagrasses, with coverage estimates ranging from small patches to coverage ranging from 600,000 km² to 1.6 million km². As foundational species, seagrasses play a critical role in maintaining coastal ecosystems, providing food for marine herbivores, carbon storage, breeding and nursery grounds for marine life, wave attenuation, and sediment stabilization. However, the rapid loss of these ecosystems highlights the urgency of advancing conservation efforts through scientific research.

A key step in safeguarding these ecosystems is sequencing their genetic blueprints to understand the traits that underlie their resilience and adaptation. To date, genomes have been generated for five species of seagrasses

including *Posidonia oceanica* (Barghini et al. 2015), *Zostera muelleri* (Golicz et al. 2015), *Zostera marina* (Olsen et al. 2016), *Cymodocea nodosa* and *Thalassia testudinum* (Ma et al. 2023). Among these, *Cymodocea nodosa* (little Neptune grass) is restricted to the Mediterranean, Black, and Caspian Seas, with an extension along the Canary Islands archipelago and the subtropical Atlantic coast of Africa. To expand genomic resources within the Cymodoceaceae family, we present the first genome for *Syringodium filiforme* (manatee grass), a pioneer seagrass native to the Atlantic Ocean. *Syringodium filiforme* is widely distributed in the coastal waters of the Gulf of Mexico, the Caribbean, and Bermuda, thriving in both shallow and deep tropical to subtropical water and recognized for its thin spaghetti-like leaves (Larkum, Orth, and Duarte 2006).

In contrast, *T. testudinum* (turtle grass) is a climax tropical seagrass with long, relatively broad leaves, unique to the greater Caribbean region (Larkum, Orth, and Duarte 2006). It is highly abundant and common throughout Caribbean islands, such as the Virgin Islands, Puerto Rico, and across the Atlantic Ocean. While the Hydrocharitaceae family already has genomic representation, expanding resources for this family is vital to understanding the diversity of strategies between climax and pioneer species. To address this, we sequenced the first genome for *Halophila stipulacea* (midrib vander grass) in the Caribbean, a small pioneer species that has become an invasive species in many regions. Native to the Indian Ocean and Red Sea, *H. stipulacea* migrated through the Suez Canal into the Mediterranean (Lipkin 1975), and over half a century later, it reached the Caribbean (Ruiz and Ballantine 2004), and recently expanded to the continental U.S. in Florida (Campbell et al. 2024). Although a raw contig level genome *H. stipulacea* has been sequenced (Tsakogiannis et al. 2020), it is not yet available on NCBI. Future comparative analyses will be crucial for gaining deeper insights into its ecological success and invasive potential.

Methods

Single wild-collected individuals were sampled for this study. Tissue was collected via snorkel in Escambron, Puerto Rico (GPS), on February 4, 2024 (DRNA permit 2024-IC-018). Seagrasses were dried. DNA extraction was performed using the Qiagen DNAeasy genomic extraction kit using the standard protocol. A paired-end sequencing library was constructed using the Illumina TruSeq kit according to the manufacturer's instructions. The library was sequenced on an Illumina Hi-Seq platform in paired-end, 2 × 150 bp format. The resulting fastq files were trimmed of adapter/primer sequence and low-quality regions with Trimmomatic v0.33 (Bolger, Lohse, and Usadel 2014). The trimmed sequence was assembled by SPAdes v2.5 (Bankevich et al. 2012) followed by a finishing step using Zanfona (Kieras, O'Neill, and Pirro 2021).

Results and Data Availability

All data, including raw reads and assembled genome sequence, is available via Genbank.

Taxa name	Genome Size	Accession no.
<i>Syringodinium filiforme</i>	1.6 Gb	GCA_047292615.1
<i>Halophila stipulacea</i>	3.2 Gb	GCA_047748335.1

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