

# Molecular Divergence of Holopelagic *Sargassum* Species Using Extensive Field Samples from the Tropical and Subtropical Atlantic

## Divergencia Molecular de las Especies Holopelágicas de *Sargassum* Mediante el Uso de Extensas Muestras del Atlántico Tropical y Subtropical

## Divergence Moléculaire D'espèces Holopélagiques de *Sargassum* à L'aide de Vastes Échantillons de l'Atlantique Tropical et Subtropical

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### EXTENDED ABSTRACT

#### Introduction

The genus *Sargassum* harbors the only holopelagic species of macroalgae in the world, *S. fluitans* and *S. natans*. Distributed throughout the Caribbean, Gulf of Mexico and North Atlantic, both species exhibit a wide range of morphological variation (Winge 1923, Parr 1939). Historically, common morphological variants of *Sargassum* included *S. fluitans III* and *S. natans I*, with greatest abundances in the Sargasso Sea and Gulf of Mexico. A third, previously-rare form, morphologically described as *S. natans VIII*, has recently inundated beaches in the tropical Atlantic and Caribbean, resulting in devastating ecological and economic impacts. These unprecedented accumulations reveal shifting patterns of *Sargassum* distribution, abundance and diversity in the Atlantic (Gower et al. 2013, Johnson et al. 2013, Schell et al. 2015). Molecular tools help resolve the genetic and evolutionary underpinnings of changes in *Sargassum* biodiversity while furthering nuanced identification of *Sargassum* morphological variants.

Sea Education Association has amassed decades of *Sargassum* observations and specimens on repeat cruise tracks in the North Atlantic and Caribbean. Here, we developed molecular tools to assess genetic divergence and differentiate among *Sargassum* forms collected at sea.

#### Methodology

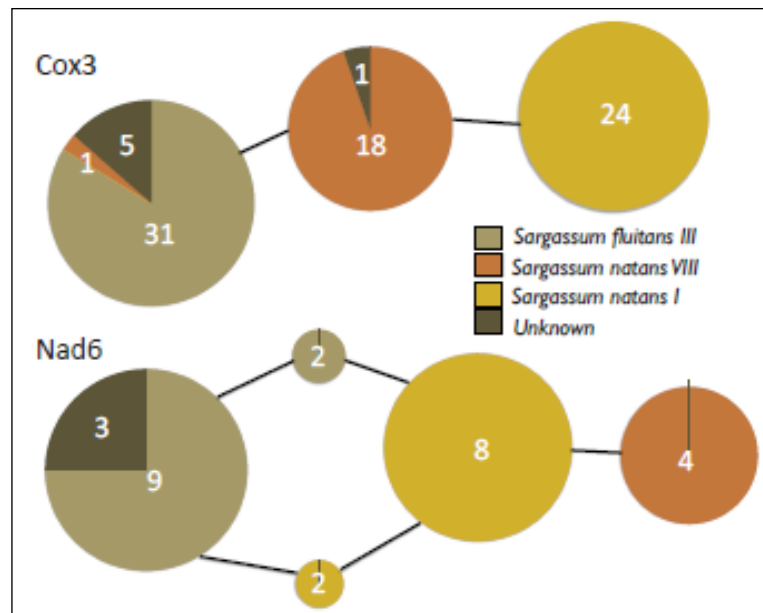
Over 200 samples of *Sargassum* were collected from the Sargasso Sea, Caribbean, equatorial and tropical Atlantic between 2016 - 2018 aboard the *SSV Corwith Cramer* during Sea Education Association's SEA Semester voyages. All specimens used in this study were first classified using traditional morphological techniques. *S. fluitans III* has thorny stems, broad blades, oblong bladders, and no spine on the bladders. *S. natans I* exhibits smooth stems, spherical bladders, thin blades, and apical spines on the bladders. *S. natans VIII* is distinguished by smooth stems, spherical bladders, broad blades, and only rarely spines on the bladders. At first glance or without examination of the stems, *S. natans VIII* is easily misidentified as *S. fluitans III*, stressing the importance of developing molecular tools to complement morphological identification of *Sargassum* forms.

We examined genetic divergence in a subset of these field samples at two mitochondrial genes, Cox3 (n = 80) and Nad6 (n=28). Cox3 and Nad6 genes were chosen because they are highly conserved and thus reliable as phylogenetic markers. Primers were designed to amplify the Cox3 and Nad6 genes using published *Sargassum* mitochondrial genomes (Amaral-Zettler et al. 2017). Nad6 and Cox3 demonstrated the potential to resolve *Sargassum* forms, but had not been previously applied in this manner. We extracted DNA from *Sargassum* specimens, amplified the selected genes using newly designed primers, and sequenced gene products using Sanger sequencing.

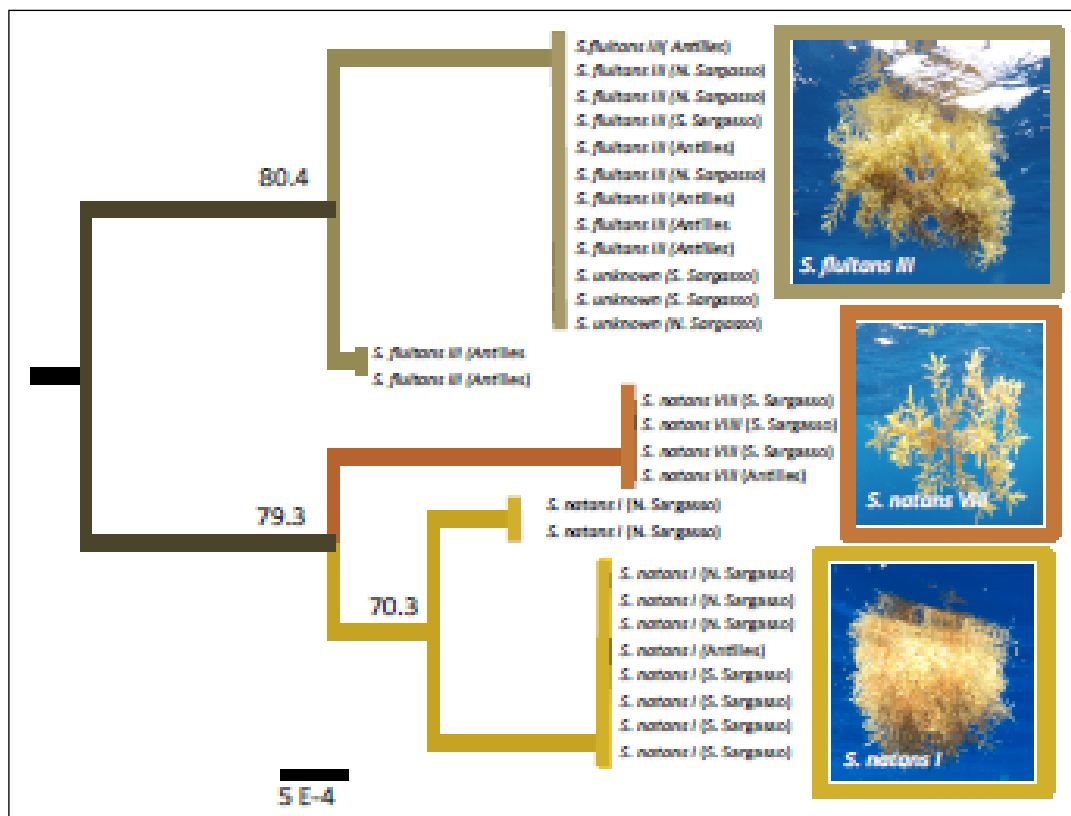
#### Results and Implications

Both Cox3 and Nad6 mitochondrial genes successfully differentiated among the three common forms of *Sargassum* using specimens collected at sea over wide geographic space and time. *S. natans VIII* exhibited 0.349% divergence from *S. natans I* (one base pair difference at both genes) and 0.698% divergence from *S. fluitans III* (one base pair difference at Cox3 and three base pair differences at Nad6), calculated using concatenated Cox3 and Nad6 sequences (Figure 1, Figure 2). Long-established as unique species, *S. natans I* and *S. fluitans III* themselves differ by just 0.698% (two base pair differences at both genes). While sequence variability within Cox3 and Nad6 mitochondrial genes is generally low, *S. natans VIII* samples exhibited unique genetic sequences that differed consistently from *S. fluitans III* and *S. natans I*, suggesting the potential need for phylogenetic reclassification.

In this study, Nad6 and Cox3 further demonstrated their ability to resolve identification of morphologically rare or ambiguous *Sargassum* samples. For example, when those specimens classified as “unknown” during initial at-sea examination (due to blade, bladder or stem damage or ambiguities) were analyzed genetically, they grouped within the genetic



**Figure 1.** Haplotype networks of Nad6 and Cox3 sequences. Circles correspond to unique haplotypes, or genetic sequence types. Color indicates *Sargassum* form and circle size is proportionate to the number of individuals sharing each haplotype. White numbers represent individuals from each *Sargassum* type sharing each haplotype. Lines represent single nucleotide differences. Nad6 and Cox3 genes reveal unique genetic sequences that distinguish among *Sargassum* forms. *S. natans VIII* represents a unique sequence type differing by one to three bases from other *Sargassum* forms at Nad6 and Cox3 genes. *S.*



**Figure 2.** A neighbor-joining-inferred phylogeny of concatenated Cox3 and Nad6 sequences from field samples of *Sargassum* collected between 2016 and 2018. Scale bar represents evolutionary distance, and numbers at the nodes represent bootstrap confidence values. *S. natans VIII* exhibits 0.349% divergence from *S. natans I*, and 0.698% divergence from *S. fluitans III*.

haplotypes of regionally-common *Sargassum* forms.

Future application of these genes may improve understanding of root evolutionary mechanisms behind shifting *Sargassum* dynamics. In particular, the recurrence of economically- and ecologically-damaging *Sargassum* inundation events across the Caribbean and tropical Atlantic demands greater knowledge of the distribution, evolution and origins of *Sargassum* diversity. We seek to use Cox3 and Nad6 genes, as well as others, to differentiate among rare and difficult to identify morphological variants within SEA's extensive archive to further resolve *Sargassum* phylogeography while concurrently developing higher-resolution molecular tools to target population-level divergence and more recent evolutionary history.

**KEYWORDS:** *Sargassum*, molecular divergence, mitochondrial genes

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