

# Genetic Variation Among Morphological Forms of Pelagic *Sargassum* and Associated Hydroids

## Variación Genética Entre las Formas Morfológicas de *Sargassum* Pelágico y Hidrozoos Asociado

### Variation Génétique Entre les Formes Morphologiques de *Sargassum* Pélagique et Hydroids Associé

AMY N.S. SIUDA<sup>1,2,\*</sup>, DEBORAH S. GOODWIN<sup>2</sup>, JEFFREY M. SCHELL<sup>2</sup>, ETHAN ALLEY<sup>2,3</sup>, DANA BLOCH<sup>2,4</sup>, SHALAGH CANNING<sup>2,5</sup>, WALTER HUTCHESON<sup>2,6</sup>, ALESIA HUNTER<sup>2,7</sup>, KATHLEEN MCKEEGAN<sup>2,8</sup>, KATE PETERSEN<sup>2,9</sup>, ROBIN PETERSEN-ROCKNEY<sup>2,10</sup>, KATHERINE RUNNING<sup>2,11</sup>, LAURA A. COONEY<sup>2</sup>, and ANNETTE F GOVINDARAJAN<sup>12</sup>

<sup>1</sup>Eckerd College, 4200 54<sup>th</sup> Avenue S, St. Petersburg, Florida 33711 USA. [siudaan@eckerd.edu](mailto:siudaan@eckerd.edu)

<sup>2</sup>Sea Education Association, P.O. Box 6, Woods Hole, Massachusetts 02543 USA.

<sup>3</sup>Harvard College, 86 Brattle Street, Cambridge, Massachusetts 02138 USA.

<sup>4</sup>Bowdoin College, Brunswick, Maine 04011 USA.

<sup>5</sup>Boston College, 140 Commonwealth Avenue, Chestnut Hill, Massachusetts 02467 USA.

<sup>6</sup>New York University, 70 Washington Square South, New York, New York 10012 USA.

<sup>7</sup>Beloit College, 700 College St., Beloit, Wisconsin 53511 USA.

<sup>8</sup>Whitman College, 345 Boyer Avenue, Walla Walla, Washington 99362 USA.

<sup>9</sup>Evergreen State University, 2700 Evergreen Parkway NW, Olympia, Washington 98505 USA.

<sup>10</sup>Oberlin College, 101 North Professor Street, Oberlin, Ohio 44074 USA.

<sup>11</sup>George Washington University, 2121 Eye Street NW, Washington, D.C. 20052 USA.

<sup>12</sup>Woods Hole Oceanographic Institution, 45 Water Street, Woods Hole, Massachusetts 02543 USA.

#### EXTENDED ABSTRACT

Pelagic *Sargassum* is comprised of two species distinguished by stem characteristics. *S. fluitans* has thorny stems and *S. natans* has smooth stems (Parr 1939). Each species is further divided into multiple morphological forms, although *S. fluitans* III Parr and *S. natans* I Parr historically dominated abundance by 90% or more (Parr 1939). Until recently, pelagic *Sargassum* observations indicated abundance maxima in the Sargasso Sea, at the center of the North Atlantic gyre, and the Gulf of Mexico (Parr 1939, Gower and King 2011, Hu et al. 2016). The Caribbean region, Brazil, and the west African coast have experienced episodic inundations of pelagic *Sargassum* since 2011. Coincident with the recent abundance increase in the Caribbean, there has been a shift in pelagic *Sargassum* diversity. In the western tropical Atlantic, eastern Caribbean and Antilles Current (north of Puerto Rico) during late 2014, Schell et al. (2015) documented mixed assemblages of pelagic *Sargassum* overwhelmingly dominated by the previously rare *S. natans* VIII Parr. However, with broad leaves and bladders often lacking spines, *S. natans* VIII Parr was mistaken for *S. fluitans* III Parr in some initial reports from the region. To compound the confusion associated with identification, *Aglaophenia latecarinata*, a hydroid epibiont that was historically exclusive to and dominant on *S. fluitans* III Parr (Calder 1995), was also dominant on *S. natans* VIII Parr.

In an attempt to validate morphological species delineations in pelagic *Sargassum* and test the hypothesis that the recent influx of *S. natans* VIII Parr originated from an alternate source region, we collected *Sargassum* and hydroid specimens from the Sargasso Sea, western tropical Atlantic and eastern Caribbean. During four separate Sea Education Association (Woods Hole, MA) cruises spanning from April 2015 through May 2016, we used both a dip net and neuston net to collect 44 pelagic *Sargassum* and 53 associated hydroid samples. We aimed to collect approximately 10 replicates at each sampling site. *Sargassum* and hydroid collections were not necessarily coincident due to distinct distribution patterns of the now three common forms of pelagic *Sargassum*. *S. natans* VIII Parr and associated hydroids were collected from the western tropical Atlantic during late 2015. *S. fluitans* III Parr samples were collected during late 2015 and early 2016 from the eastern Caribbean. Hydroids associated with *S. fluitans* III Parr were collected from the Antilles Current during April 2016. Additional hydroids were isolated in May 2016 from *S. natans* VIII Parr and *S. fluitans* III Parr collected simultaneously from the northern Gulf Stream. *S. natans* I Parr was collected from the Sargasso Sea in April and May 2015. Pelagic *Sargassum* abundance was extremely low in the Sargasso Sea during spring 2016, hence the lack of additional *S. natans* I Parr samples from this period. *Sargassum* was dried in silica gel and hydroid colonies were preserved in 95% ethanol prior to DNA extraction. We amplified DNA segments from three target genes for *Sargassum* and two target genes for the hydroid. For *Sargassum*, we sequenced the mitochondrial cytochrome oxidase 3 subunit (cox3), the Ribulose biphosphate carboxylase large chain + spacer + partial Ribulose biphosphate carboxylase small chain (rbcL-S), and the mitochondrial intergenic spacer region (mtsp) between the mitochondrial 23S gene and transcription ribonucleic acid Valine (tRNA-Val). For hydroids, we sequenced mitochondrial 16S rDNA and cytochrome c oxidase I (COI) markers. After assembly and alignment of bi-directional sequences, we created neighbor-joining trees and haplotype networks to visualize the relationships between pelagic *Sargassum* forms as well as hydroids collected from each of the pelagic *Sargassum* substrates.

Sequences were identical across samples of individual pelagic *Sargassum* forms. *S. fluitans* III Parr sequences for all three markers were distinct from those of the *S. natans*-complex (Figure 1). There were ten polymorphic loci in the mtsp

region as well as two polymorphic loci and nine base pair insertion in *rbcl-S* region. With the present set of genetic markers, we could not distinguish between *S. natans* I Parr and *S. natans* VIII Parr despite clear morphological differences. Interestingly, we came across a few samples of what we provisionally identified as *S. natans* II Parr; these samples were genetically distinct from both the *S. natans*-complex and *S. fluitans* III Parr (Figure 1). *A. latecarinata* sequences clustered into two distinct groups according to *Sargassum* substrate, even for samples collected from co-occurring *S. natans* VIII Parr and *S. fluitans* III Parr in the northern Gulf Stream (Figure 2). In the case of the mitochondrial 16S rRNA encoding region, we identified four distinct haplotypes with a maximum of a three base pair difference between sequences. Four distinct haplotypes were identified among sequences from the mitochondrial CO1 region, including one haplotype that represented a single hydroid sample collected from *S. natans* II Parr.

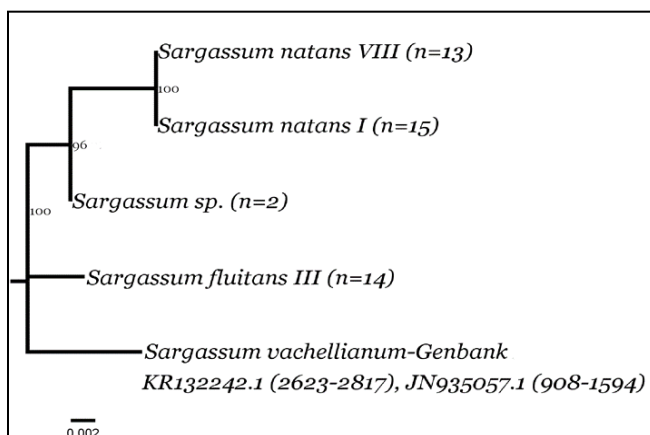
These preliminary genetic results support the morphological identification of *S. natans* VIII Parr by Schell et al. (2015) and the physical evidence for an alternate pelagic *Sargassum* source region associated with the recent inundations of *S. natans* VIII Parr (Gower et al. 2013; Franks et al. 2016). *S. natans* VIII Parr is genetically distinct from *S. fluitans* III Parr, despite superficial morphological similarities, and genetically indistinct, using the selected marker genes, from *S. natans* I Parr. Moreover, the associated hydroids were genetically distinct between *Sargassum* substrates. This result was anticipated, as *A. latecarinata* produces a planula larva that settles quickly and is dependent upon a substrate for dispersal. Together, these complementary genetic results reinforce the previous morphology-driven assertion that recent inundation events introduced a heretofore rare form of pelagic *Sargassum* to the Caribbean region that likely originated from the

equatorial North Atlantic. This shift in regional *Sargassum* abundance and diversity not only has implications for coastal ecosystems and economies experiencing inundations, but also for pelagic populations that utilize *Sargassum* as a nursery or foraging habitat. A deeper examination of genetic as well as species and functional diversity among the *S. natans* VIII Parr associated community of organisms is warranted.

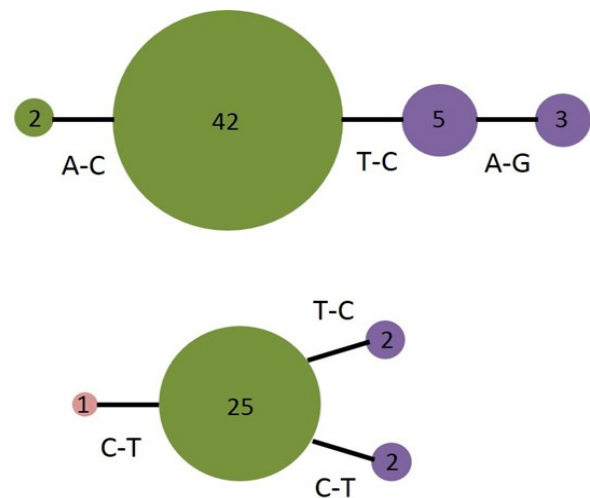
**KEYWORDS:** *Sargassum*, *Aglaophenia latecarinata*, hydroid, population genetics, Caribbean

#### LITERATURE CITED

- Calder, D.R. 1995. Hydroid assemblages on holopelagic *Sargassum* from the Sargasso Sea at Bermuda. *Bulletin of Marine Science* 56(2):537-546.
- Franks, J.S., D.R. Johnson, and D.S. Ko. 2016. Pelagic *Sargassum* in the tropical North Atlantic. *Gulf and Caribbean Research* 27(1):SC6-SC11.
- Gower, J.F.R. and S.A. King. 2011. Distribution of floating *Sargassum* in the Gulf of Mexico and the Atlantic Ocean mapped using MERIS. *International Journal of Remote Sensing* 32(7):1917-1929.
- Gower, J., E. Young, and S.A. King. 2013. Satellite images suggest a new *Sargassum* source region in 2011. *Remote Sensing Letters* 4(8):764-773.
- Hu, C., B. Murch, B.B. Barnes, M. Wang, J-P Maréchal, J. Franks, D. Johnson, B. Lapointe, D.S. Goodwin, J.M. Schell, and A.N.S. Siuda. 2016. *Sargassum* watch warns of incoming seaweed. *Eos* 97. <http://doi:10.1029/2016EO058355>.
- Parr, A. 1939. *Quantitative Observations on the Pelagic Sargassum Vegetation of the Western North Atlantic*. Bulletin of the Bingham Oceanographic Collection, Vol. VI. Peabody Museum of Natural History, Yale University, New Haven, Connecticut USA. 93 pp.
- Schell, J.M., D.S. Goodwin, and A.N.S. Siuda. 2015. Recent *Sargassum* inundation events in the Caribbean: shipboard observations reveal dominance of a previously rare form. *Oceanography* 28(3):8-10.



**Figure 1.** Consensus Neighbor Joining Tree of the concatenated *rbcl-S* and *mtsp* loci. Bootstrap support is indicated at nodes, with those less than 95% supported collapsed. Number of replicates of each sequence shown in parentheses. Branch length is unconstrained and corresponds to genetic distance at a scale of 0.002. Outgroup sequences obtained from GenBank using NCBI Blast. Using morphological methods, *Sargassum* sp. has been provisionally identified as *S. natans* II Parr.



**Figure 2.** Haplotype networks of the mt16S (top) and CO1 (bottom) genes in *A. latecarinata*. Circles and associated values depict number of samples within each distinct haplotype. Unbroken lines connecting circles represent a single nucleotide difference. Nucleotide changes are noted below each connecting line. Sequences from hydroids found on *S. natans* VIII Parr are in green, *S. fluitans* III Parr are in purple, and *S. natans* II Parr are in pink.