Molecular and morphological analysis of *Sargassum* species from San Salvador, the Bahamas

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Introduction

The genus *Sargassum* is a group of brown algae in the order Fucales, which includes the only truly pelagic macroalgal species. *Sargassum* is an important nursery and habitat in the low-nutrient, open water of the Sargasso Sea (Franks et al., 2016). Recently, *Sargassum* blooms have become more frequent in the Caribbean and scholars have done genomic research and molecular assessments to try to identify the species. Amaral-Zettler et al. (2017) have credited the formation of the blooms to the pelagic species *S. natans* (Form VIII) and *S. fluitans*. Work has been conducted to establish the origin of the *Sargassum* blooms. Models were used to simulate how *Sargassum* traversed to the Caribbean. Results showed that it was unlikely that the *Sargassum* originated from the Sargasso Sea, but instead consolidated around North-Eastern Brazil (Franks et al., 2016). It is important to identify the *Sargassum* found on San Salvador to ascertain if a bloom forming species is present, and if more attention is needed for potential future blooms.

Study Objective:

- Identify the *Sargassum* species found in San Salvador, the Bahamas to ascertain if bloom-forming species, *S. natans* and *S. fluitans*, are present.

Materials and Methods

Thirty samples were collected from eight sites in San Salvador, the Bahamas (Figure 5). Specimens were preserved as herbaria presses, in ethanol for morphological analysis, and in silica gel for DNA extraction.

Genomic DNA was extracted using a Qiagen DNeasy Plant Mini Kit. PCR was used to amplify the gene region cytochrome oxidase subunit 3 (cox3) with two primer sets including: forward primers cox3-467F and CAF4A along with the reverse primers cox3-901R and CAR4A (Amaral-Zettler et al., 2017; Camacho et al., 2015). The Internal Transcribed Spacer 2 (ITS-2) was also amplified using the forward primer 5.8S-BF and the reverse primer 25BR-2 (Yoshida et al., 2000).

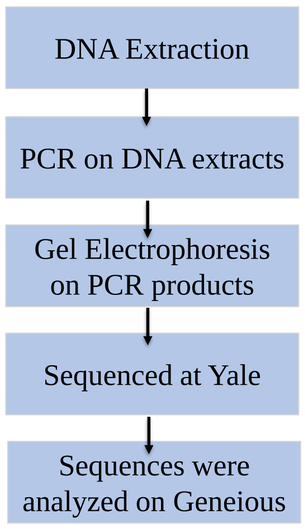

Figure 1. Flow chart of the methods that were used on the samples.

Morphological Analysis

Cross-sections were made on the blades of the specimens and observed under a compound light microscope. The presence or absence of spines on vesicles or the axis was observed to differentiate *S. natans* and *S. fluitans* (Amaral-Zettler et al., 2017).

Literature Cited

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Results

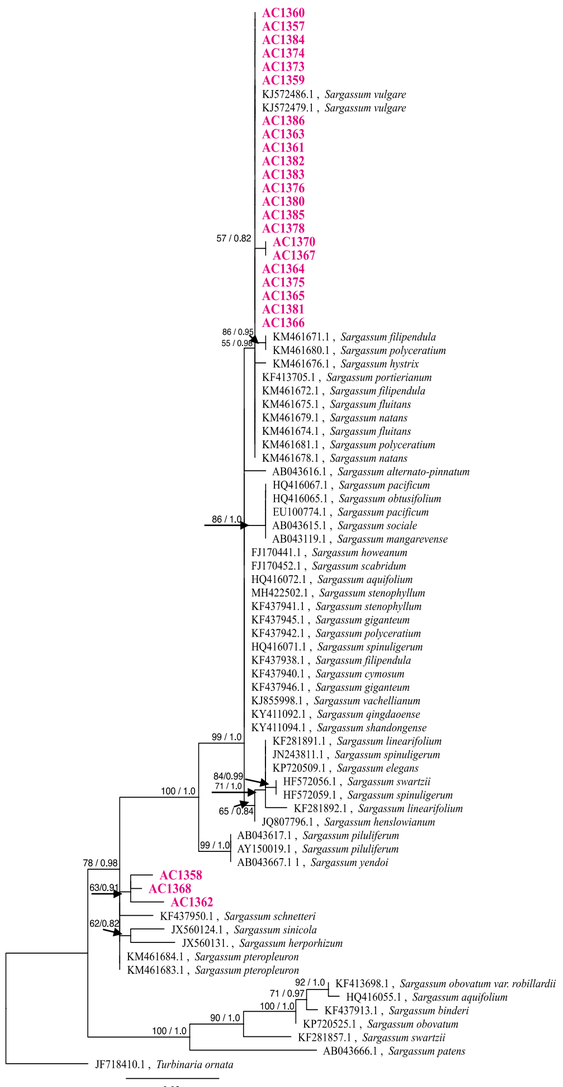

Figure 2. Phylogenetic tree of ITS-2 data with maximum likelihood bootstrap values and Bayesian posterior probabilities. Samples from this study are highlighted in color.

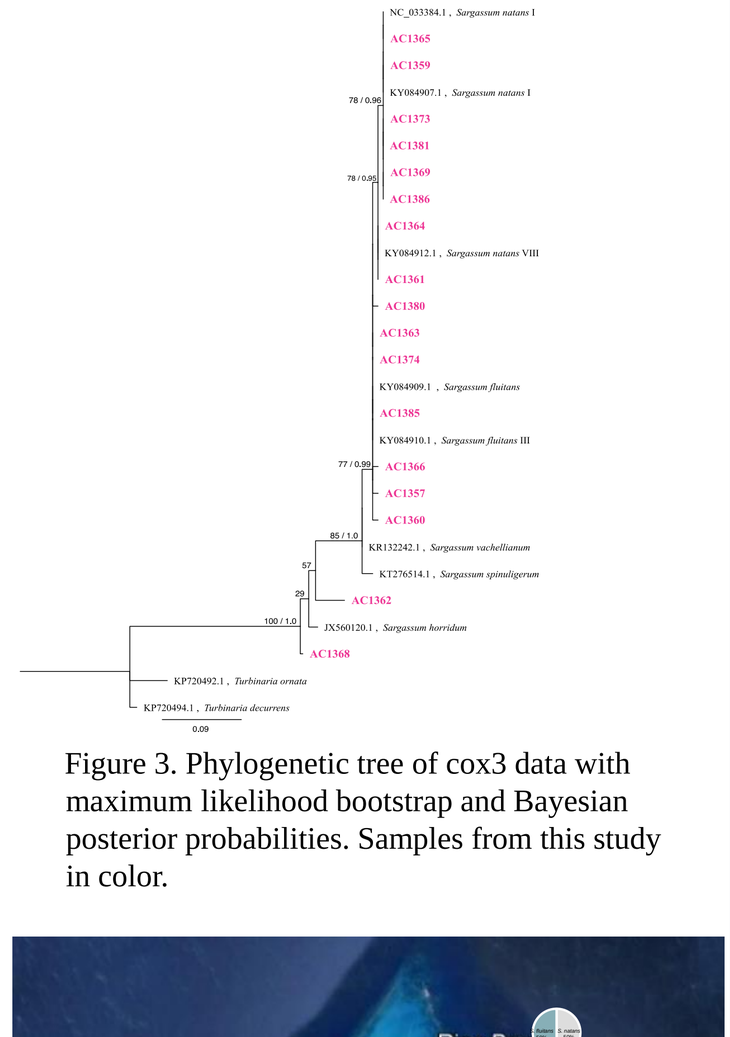

Figure 3. Phylogenetic tree of cox3 data with maximum likelihood bootstrap and Bayesian posterior probabilities. Samples from this study in color.

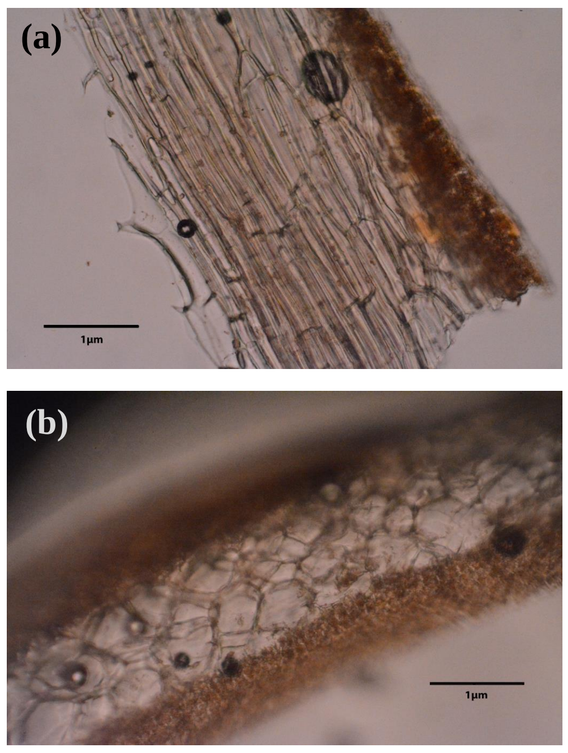

Figure 4. Cross-sections of leaves found on (a) *S. natans* and (b) *S. fluitans*.

Figure 5. Map of San Salvador with labeled sites including pie charts with the relative abundance of molecularly identified species.

Discussion

Of the total thirty samples, twenty-nine were successfully sequenced through use of the ITS-2 marker; however, this marker did not distinguish species as most of the samples were placed into a polytomy (Figure 2). Camacho et al. (2015) also reported low sequence variation in this marker as *Sargassum sp.* exhibit low genetic variability as they diversified recently (Mattio et al., 2008). *S. natans* and *S. fluitans* are only different by one base pair (Amaral-Zettler et al., 2017).

For the cox3 marker, seventeen of the thirty samples were successfully sequenced. The cox3 marker was successful in distinguishing the *S. natans* forms from *S. fluitans.* Samples identified in the field to be *S. natans* (Form I) were confirmed and were all grouped together with the reference sequences (Figure 3). The sample morphologically identified as *S. fluitans* had a base pair difference from the reference sequences, which may be a result of messy sequence data (Figure 3). Two samples were identified as *S. natans* (Form VIII), but they did not match morphologically or by habitat (Figure 3). This may present problems with the marker used to differentiate *Sargassum* species.

S. natans (Form I) was abundant and found at all the sites around San Salvador. *S. fluitans* was only found at Rice Bay, which is closest to the Sargasso Sea (Figure 5). The cross-sections made showed a distinguishable difference in the cortex structure between *S.natans* (Figure 4a) and *S. fluitans* (Figure 4b). Observations of the herbarium presses showed that *S. natans* (Form I) had spines on the vesicles, *S. fluitans* had thorns on the axis, and *S. natans* (Form VIII) had neither features (Amaral-Zettler et al., 2017). This confirmed the molecular data.

Conclusions

- *S. natans* (Form I) and *S. fluitans* are present on San Salvador, the Bahamas. The bloom-forming species *S. natans* (Form VIII) was not present on the island; however, an abundance of *S. natans* (Form I) was present and can potentially form blooms.
- Some primers were not useful in delimiting *Sargassum* species.
- It is essential to pair molecular and morphological work to identify *Sargassum* species.

Future Work

- Collect more samples from different areas in the Caribbean to see if bloom-forming species are present in more areas.
- Design more effective primers to delimit species within *Sargassum*. Amaral-Zettler et al., (2017) studied whole mitochondrial genomes to design new primers.