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

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The genus *Sargassum* is a group of brown algae in the order Fucales, which includes the only truly pelagic macroalgal species. Recently, *Sargassum* blooms have become more frequent in the Caribbean where scholars have credited the formation of the blooms to the pelagic species *S. natans* (Form VIII) and *S. fluitans* (Amaral-Zettler et al., 2017). The objective of this study was to determine if bloom forming *Sargassum* species are present on San Salvador, the Bahamas. A total of thirty samples were collected from eight sites around the island. Samples 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. Polymerase chain reaction (PCR) was used to amplify the mitochondrial 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 nuclear gene region, 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). After confirming PCR product size using gel electrophoresis, samples were then cleaned with ExoSAP and sent to Yale for sequencing. Sequences were then analyzed on GeneiousR9 (https://www.geneious.com). Cross-sections were made on the blades of the preserved samples for morphological analysis. The

presence or absence of spines on vesicles or the axis was observed on the herbaria presses to differentiate *S. natans* and *S. fluitans* (Amaral-Zettler et al., 2017).

Of the total thirty samples, twenty-nine were successfully sequenced using the ITS-2 marker, while only seventeen were successfully sequenced with the cox3 marker. The ITS-2 marker did not delimit *Sargassum* species well as most of the samples were placed into a polytomy. Low sequence variation in this marker may be due to low genetic variability within the genus, as they have diversified recently (Mattio et al., 2008). The cox3 marker was successful in delimiting the *S. natans* forms from *S. fluitans*. Six samples were identified to be *S. natans* (Form I). Two samples were identified to be the bloom former *S. natans* (Form VIII); however, these samples did not match morphologically or by habitat. 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. The cross-sections made showed a distinguishable difference in the cortex structure between *S.natans* and *S. fluitans*. 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. *S. natans* (Form I) was abundant and found at all the sites around San Salvador, which may result in potential blooms. Future work may benefit studies of whole genomes as *Sargassum sp.* sequences are very similar. More effective primers may also be necessary.

**Works Cited**

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