

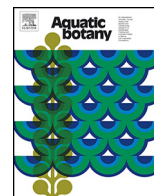


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# A persistent bloom of *Anadyomene* J.V. Lamouroux (Anadyomenaceae, Chlorophyta) in Biscayne Bay, Florida

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### ABSTRACT

Green macroalgal blooms are becoming a common problem in coastal waters and estuaries. This study describes the first occurrence of a persistent macroalgal bloom of the genus *Anadyomene* J.V. Lamouroux (Cladophorales, Anadyomenaceae) in the world and particularly in Biscayne Bay, FL, USA. The morphological-based identification of species was verified by a molecular analysis that sequenced the variable C1D2 region of the large subunit (LSU) nrDNA. Results indicate that the bloom is composed of two species: *Anadyomene stellata*, reported previously for Florida, and a diminutive perforate undetermined species, *Anadyomene* sp., potentially representing an introduction in the area. General surveys in Biscayne Bay based on a stratified random design, to visually estimate the percent cover of submerged aquatic vegetation, date from 1999; using the same methods recent intensive surveys of the detected bloom were conducted once a year from 2010 to 2012. Results show that the *Anadyomene* bloom densities have persisted since 2005 through 2012 covering an area of approximately 60 km<sup>2</sup> of seagrass habitats. The spatial distribution of the bloom is restricted to the central inshore section of the Bay, an area affected by canals and groundwater discharges. The persistent 75% cover reported for several sites, has caused significant negative impacts to seagrass beds. This bloom occurring adjacent to metropolitan Miami, adds to the world trend of increasing green macroalgal blooms occurring at enriched coastal waters.

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### 1. Introduction

Harmful algal blooms (HAB) are becoming an increasingly significant problem as population and coastal development continue to grow (Ye et al., 2011). The problem is so prevalent that almost every country with a coastline has either experienced a bloom or is facing the risk of one (Anderson, 2009). Algal blooms are generally associated with phytoplankton (microalgae and cyanobacteria) species, however, several macroalgal species are also known to bloom (Valiela et al., 1997; Smith et al., 2005; Piñón-Gimate et al., 2009; Nelson et al., 2008). These ephemeral or persistent HABs have been associated with nutrient loads from urban or agricultural drainages and other sources such as shrimp farms (Valiela et al., 1997; Piñón-Gimate et al., 2009). Introduced algal species

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have been also associated with blooms, such as *Caulerpa taxifolia* (M. Vahl) C. Agardh in the Mediterranean or *Caulerpa brachypus* f. *parvifolia* (Harvey) A.B. Cribb in Florida, making the identification of any algal bloom species a very important step for its management (William and Smith, 2007; Lapointe and Bedford, 2010; Guidone and Thornber, 2013).

Macroalgal blooms have been reported to be composed of red, green and brown algae (Valiela et al., 1997; William and Smith, 2007); however the majority and largest blooms are found within species of the Chlorophyta phylum. Some “green tides” reported belong to the Ulvophyceae, including several species of the genera *Ulva* Linnaeus and *Monostroma* Thuret. The largest macroalgal bloom reported so far is the *Ulva prolifera* O.F. Müller 2008 bloom offshore of Qingdao, China. This bloom which span was approximately 13,000 km<sup>2</sup> in the Yellow Sea produced approximately 20 million wet tons of algae with an extremely expensive management cost (Leliaert et al., 2009; Liu et al., 2009). Other examples of blooming-forming species of the class Ulvophyceae include temperate species such as *Cladophora vagabunda* (Linnaeus) Hoek in Waquoit Bay, MA, USA (Peckol et al., 1994); and tropical species such as *Cladophora prolifera* (Roth) Kützing in Bermuda which lasted for several decades (Bach and Josselyn, 1978; Lapointe and O’Connell, 1989). Recent reports include *Anadyomene gigantodyction* Littler and Littler, found overgrowing deep water gorgonian corals in Belize (Littler and Littler, 2012) and *Boodlea composite* (Harvey) F. Brand at the Northwestern Hawaiian Islands (Vroom et al., 2009). All the above mentioned species have in common a morphology and anatomy that results in a high surface area to volume ratio (SA:V) that suggests they can respond rapidly to increased nutrient inputs (Littler and Littler, 1980).

Biscayne Bay is a subtropical estuarine lagoon within Miami-Dade County, FL, and adjacent to the City of Miami, which has approximately 450,000 inhabitants, with 2.5 million inhabitants in the county. Since early 1950s, the hydrology of the bay has been altered to meet the needs of the growing population and has become subject to dredging, urban runoff, sewage, and other anthropogenic influences that have altered the health and function of the ecosystem (Light and Dineen, 1994). In addition, the port of Miami, located in Biscayne Bay, has a large cargo and tourism traffic increasing the likelihood of species introduction in the area.

The submerged aquatic vegetation (SAV) of the lagoon is characterized by seagrasses and drifting and rhizophytic macroalgae, with approximately 60 species reported; including one species of *Anadyomene* (Biber and Irlandi, 2006; Lirman et al., 2008; Collado-Vides et al., 2011). Biscayne Bay has the lowest water column nutrient concentrations in the state, however, specific regions of Biscayne Bay are reported to have higher water-column nutrients indicative of terrestrial/groundwater nutrient loading (Caccia and Boyer, 2007; Stalker et al., 2009). These conditions are consistent with the high nitrogen (N) content found in seaweed tissue reported by Collado-Vides et al. (2011), which are similar to/or higher than those found in algae under experimentally enriched treatments, as well as those growing close to nutrient-rich mangrove areas (Lapointe, 1987, 1997), suggesting that SAV organisms are growing exposed to relatively high nutrient availability indicating elevated risk levels for blooming events.

Consistent with these nutrient patterns, we report the first occurrence of a persistent bloom of *Anadyomene* V.J. Lamouroux (Cladophorales, Anadyomenaceae) species. Morphological and phylogenetic molecular analyses were used to test the taxonomic identity of the blooming species, which has the potential of being a new record for the study site. Due to the presence of canals, the highest abundance and persistence of the bloom is expected to be close to areas affected by canals discharges compared with areas further offshore. The information included in this study adds to the increasing list of green macroalgal blooms in coastal habitats,

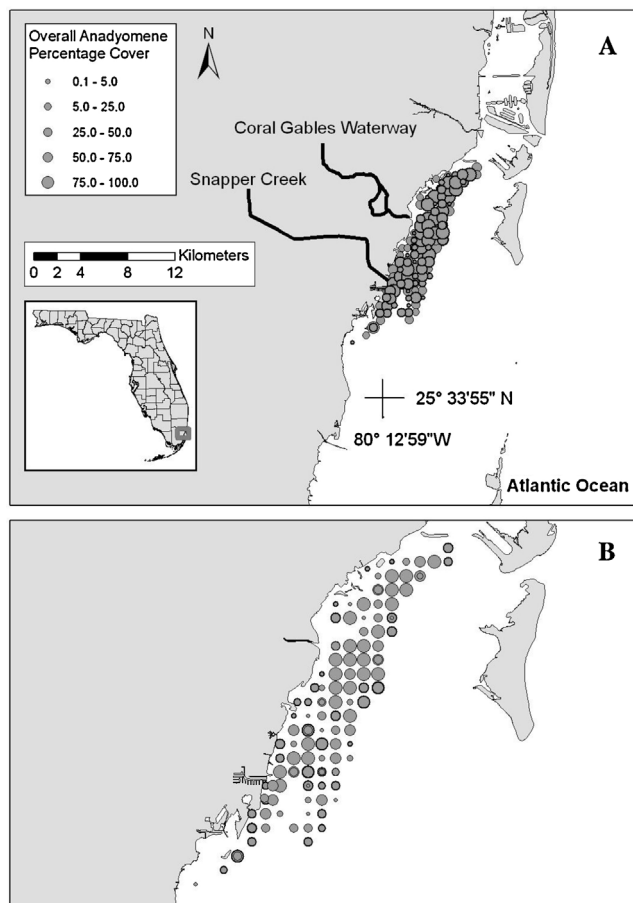


Fig. 1. Map of Biscayne Bay showing the distribution of the algal bloom. (a) General geographical location of the bloom, (b) zoom of the bloom abundance distribution.

and call the attention to shifting conditions in coastal waters with potential negative consequences on the structure and function of seagrass habitats.

## 2. Methods

### 2.1. Study area

Biscayne Bay is adjacent to the city of Miami, FL, USA (25° 33' 57" N, 80° 12' 59" W) (Fig. 1). Due to its natural and economic importance, Biscayne Bay is designated an Outstanding Florida Waterway by the state of Florida. Biscayne Bay is comprised of two state aquatic preserves and a national park. Biscayne National Park is located in the central part of the bay and is managed by the National Park Service. The Biscayne Bay Aquatic Preserves are located to the north and south of the national park, where the bloom is most dense, and is managed by the Florida Department of Environmental Protection. The lagoon is a coastal estuary affected by freshwater and nutrient inputs from canals, groundwater, precipitation, and overland flows (Caccia and Boyer, 2007; Stalker et al., 2009). The submerged aquatic vegetation of Biscayne Bay is characterized by four seagrass species with the dominance of *Thalassia testudinum* Banks ex König, followed by *Halodule wrightii* Ascherson, and *Syringodium filiforme* Kützing. *Ruppia maritima* Linnaeus has a limited presence, reduced to areas with high fresh water loads. Approximately 60 species of macroalgae have been reported within Biscayne Bay, with a dominance of species in the genera *Halimeda* J.V. Lamouroux and *Penicillus* Lamarck within seagrass beds under marine conditions, and red algae of the genus *Laurencia* J.V.

Lamouroux within seagrasses exposed to lower and more variable salinity (Collado-Vides et al., 2011). The bottom where the bloom was found is dominated by *T. testudinum* and algae of the genus *Halimeda* and *Penicillus*.

## 2.2. Morphological identification

Algal material was collected at the bloom sites, and preserved in 4% formaldehyde seawater solution for identification. Taxonomic characteristics were evaluated following Littler and Littler (1991). Live material was collected, and rapidly dried in 20 ml vials with silica gel for molecular analysis; some live material was kept fresh and used to measure cell dimensions and take photographs. Microscopic pictures were taken with an eye piece camera ScopeTech (Hangzhou, China), using a Leica microscope. Measurements were made with a calibrated ocular micrometer.

## 2.3. Molecular phylogeny

Morphology-based species identification was verified by sequencing the variable C1D2 region of the large subunit (LSU) nrDNA of two *Anadyomene* specimens from the blooming area in Biscayne Bay, in addition to seven additional specimens of *Anadyomene pavonina* from Panama, and *A. stellata* from Panama, Costa Rica, and Greece. DNA extraction, polymerase chain reaction (PCR) amplification and sequencing were performed as described in Leliaert et al. (2007a). The newly generated sequences are deposited in EMBL/GenBank under accession numbers HF936684–HF936692. These sequences were then combined with available sequences from the *Anadyomene/Microdictyon* clade (Leliaert et al., 2007b), and aligned using MUSCLE (Edgar, 2004). Selection of the model of nucleotide substitution was performed using the Akaike information criterion with jModelTest v0.1.1 (Posada, 2008). Maximum likelihood (ML) phylogenetic analysis was performed using PhyML under a general time-reversible model with gamma distribution split into four categories and no separate rate class for invariable sites (GTR+G). The reliability of internal branches was evaluated with nonparametric bootstrapping (1000 replicates). The tree was rooted with “*Boodlea vanbosseae*” following Leliaert et al. (2007b).

## 2.4. Field surveys

### 2.4.1. History and abundance

Benthic habitats of Biscayne Bay have been monitored for more than 3 decades by Miami-Dade County. Systematic monitoring cover started in 1999 and 2003, by Miami Dade County and the University of Miami, respectively, conducting surveys based on a stratified random design, to visually estimate the percent cover of SAV. The percent cover of seagrass and green macroalgae was estimated for each site using *in situ* 0.25 m<sup>2</sup> quadrats (Miami Dade County, *n*=4 quadrats per site) or photographs (University of Miami, 10 images per site) (Lirman et al., 2008). Starting in 2010, an intensive monitoring program was concentrated on the area of Biscayne Bay where the algal bloom was dominant. These targeted surveys covered approximately 70 km<sup>2</sup> (Fig. 1), a total of 181 sites were surveyed in 2010–2012. Sites were aggregated in five groups based on geographic characteristics, group 1 included all sites in proximity to canal areas, group 2 included sites in the central part of the bloom, group 3 clumped sites at the edge of bloom on the central area, group 4 contained the sites at the south edge of the bloom and group 5 clumped sites in the north area of the bloom.

### 2.4.2. Statistical analysis

The 2010–2012 surveys resulted in a very large data-set, robust enough to apply parametric tests even if they do not comply with

normality and homogeneity of variance (Underwood, 1997). A multiple ANOVA followed by a *post hoc* Bonferroni multiple comparison test was applied to detect changes on species abundance, through time and space for all data from 2010 through 2012, with time (years), groups (close to canal, central, north, south edge and central edge) and time\*group as factors. A one-way ANOVA was applied for each area (groups) with year as a factor to detect area-specific changes through time. A Pearson correlation was applied to all percent cover data to evaluate relationships between *Anadyomene* sp. and *T. testudinum*. All statistical analyses were conducted in SPSS v. 19.

## 3. Results

Two species were observed forming the bloom; one species is a sturdy eperforate blade form, while the second species is a diminutive perforate form. The bloom was found in shallow waters between 2 and 4 m deep, and specimens were usually observed drifting or entangled on seagrass (Fig. 2a).

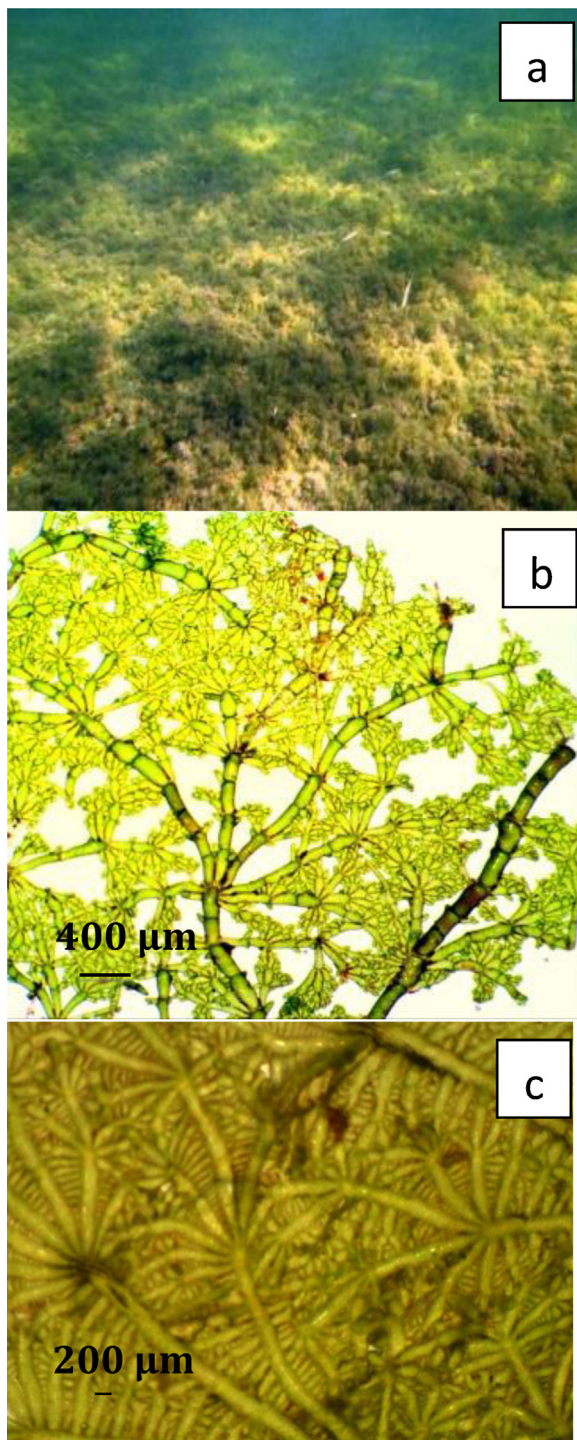
### 3.1. Morphological analysis

The diminutive form of *Anadyomene* covered large areas of seagrass beds and, in some sites, completely overgrew the seagrass shoots (Fig. 2b, Fig. A1). This species has a foliose, highly perforate mesh-like, and decumbent thallus, up to 5 cm in length and 3 cm wide. Veins are composed of uniseriate chains of cylindrical cells, decreasing in length distally; proximal vein cell dimensions are highly variable with cell length 0.66–1.1 mm, cell width 0.13–0.23 mm, and cell length/width (*l/w*) ratio 3–8; mid-blade vein cell dimensions are less variable with cell length 0.65–0.97 mm, cell width 0.13–0.19 mm width, and cell *l/w* ratio 3.8–7; distal vein cell dimension are smaller with cell length 0.32–0.57 mm, cell width 0.11–0.18 mm, and cell *l/w* ratio 2.1–3.5; veins branch polychotomously with 3–6 branches radiating peripherally throughout the blade. Interstitial cells are ovoid or elliptical, arranged randomly and branching polychotomously; little or no overlap of adjacent cells throughout the blade, blunt attachment of interstitial cells with adjacent cells that lack tentacula. Most specimens unattached, with no clear stipe formed from descending rhizoids, but rhizoidal elongations are present. The distal margin has terminal marginal cells that can be free or anastomosing to adjacent cells. Our specimens lack distinct veins bordering perforations. The morphological characteristics do not fit completely with any described species therefore we leave it as undetermined.

The larger *Anadyomene* form was also found covering large extensions of seagrass beds (Fig. 2c, Fig. A2). It had an erect foliose thallus, up to 8 cm tall, and grew as sturdy fronds forming dense clumps; blades were eperforate and robust. Veins are composed of single uniseriate chains, with cylindrical cells slightly swollen at their distal ends. The veins decrease in size toward distal margin; proximal veins cell length 1–1.8 mm, cell width 0.14–0.27 mm, cell *l/w* ratio 5.4–11.2; mid-blade cell length 0.68–1.15 mm, cell width 0.12–0.22 mm, cell *l/w* ratio 3.8–8.1, distal cell length 0.38–0.98 mm, cell width 0.13–0.21, cell *l/w* ratio 2.7–4.6; interstitial cells generally arranged parallel to each other; rhizoids mainly in the basal regions of blades; veins polychotomously branching (2–6 branches); growing edge smooth or lobed, composed of small round or oval cells. The morphological characteristics of these specimens fit the description of *Anadyomene stellata* (Wulfen) C. Agardh.

### 3.2. Molecular analysis

The partial large subunit (LSU) rDNA alignment, containing 41 sequences of the *Anadyomene/Microdictyon* clade was 630 sites long, including 56 variable sites. The maximum likelihood tree is



**Fig. 2.** (a) blooming area reaching 100% cover displacing seagrass, (b) *Anadyomene* sp. habit, (c) *A. stellata* habit.

shown in Fig. 3. The sequence data showed that the two specimens from the bloom belong to two different species. One sequence clusters with specimens identified as *A. stellata* from the Mediterranean Sea, Canary Islands and the Caribbean Sea. *A. stellata* was sister to *Anadyomene saldanhae* Joly et Oliveira Filho also including specimens from both sides of the Atlantic Ocean. The other specimen, morphologically undetermined, formed a singleton that was most closely related to the *A. stellata*–*A. saldanhae* clade.

**Table 1**

Two-Way ANOVA for all 2010–2012 data, group, year and group\*year as factors. Asp, *Anadyomene* sp.; As, *Anadyomene stellata*; Hw, *Halodule wrightii*; Sf, *Syringodium filiforme*; Tt, *Thalassia testudinum*. Superscript numbers by species represent Bonferroni significant differences.

Factor	Species	df	Mean square	F	p
Group	Asp <sup>3-4</sup>	4	83337.856	85.009	<0.0001
	As <sup>1-2-3-4</sup>	4	4338.549	20.280	<0.0001
	Hw <sup>3</sup>	4	560.864	18.314	<0.0001
	Sf <sup>3</sup>	4	1111.033	7.383	<0.0001
	Tt <sup>3-4-5</sup>	4	15889.045	22.088	<0.0001
Year	Asp <sup>1-3</sup>	2	23464.859	23.935	<0.0001
	As <sup>1-2</sup>	2	689.967	3.225	0.040
	Hw	2	52.430	1.712	0.181
	Sf <sup>2-3</sup>	2	144.960	0.963	0.382
	Tt <sup>1-2-3</sup>	2	6896.595	9.587	<0.0001
Group*year	Asp	8	6372.463	6.500	<0.0001
	As	8	458.007	2.141	0.030
	Hw	8	161.294	5.267	<0.0001
	Sf	8	90.027	0.598	0.780
	Tt	8	2303.524	3.202	0.001

### 3.3. Bloom abundance spatial and temporal distribution

The dominant SAV species observed in the region of the algal bloom within Biscayne Bay were *T. testudinum* (average cover of 26%), followed by *S. filiforme* (average cover of 4%), and *H. wrightii* (average cover of 1%). Macroalgal species were rare and represented by species of the genus *Halimeda* and *Penicillus*, occasional drifting red masses composed by species of the *Laurencia* complex could be found in areas boarding the bloom region. The overall area was dominated by the two blooming *Anadyomene* species; with an overall average cover of 42%.

*Anadyomene* species have been detected in low abundance within the Bay since the beginning of the surveys (1999), and the majority of the sites surveyed during the following years (1999–2004) had a % cover between 0% and 50%. Very few sites showing a % cover >50%. The opposite pattern was detected for the dominant seagrass, *T. testudinum*, which had in many sites a % cover >75% during the 1999–2004 period. During the years of 2005–2008 the *Anadyomene* bloom was concentrated at the north-central inshore section of the Bay, covering an area of approximately 60 km<sup>2</sup>. In that same period the abundance of the bloom increased considerably reaching 100% cover in several sites (Fig. 4). The cover of *Anadyomene* sp. and *T. testudinum* showed an inverse relationship over time, where the cover of *T. testudinum* was high in the period between 1999 and 2004, but decreased significantly between 2005 and 2008. Conversely, the abundance of *Anadyomene* sp. was low during 1999–2004, and increased significantly during 2005–2008.

The intensive three year surveys in the bloom area showed that the overall abundance of the three seagrasses and the two *Anadyomene* species had a characteristic spatial distribution with significant differences between areas. *Anadyomene* sp. had the highest abundances close to canals, in the center of the bloom and in the north area, while, *A. stellata* was concentrated at the north of the bloom. *T. testudinum* was abundant at the edges of the bloom, while *S. filiforme* and *H. wrightii* had an insignificant presence at the central edge of the bloom, with minimal abundance in the rest of the study area (Fig. 5, Table 1).

Overall abundance of *Anadyomene* sp., and *T. testudinum* had significant variability through time (Fig. 6, Table 1). A significant decrease of abundance was detected for *Anadyomene* sp. from 2011 to 2012 (Table 1) and an increase of *T. testudinum* by 2012. No significant differences between years were found for *S. filiforme* and *H. wrightii* abundance (Table 1); however the variability in abundance was different per area (Fig. 7, Table 2). The area close

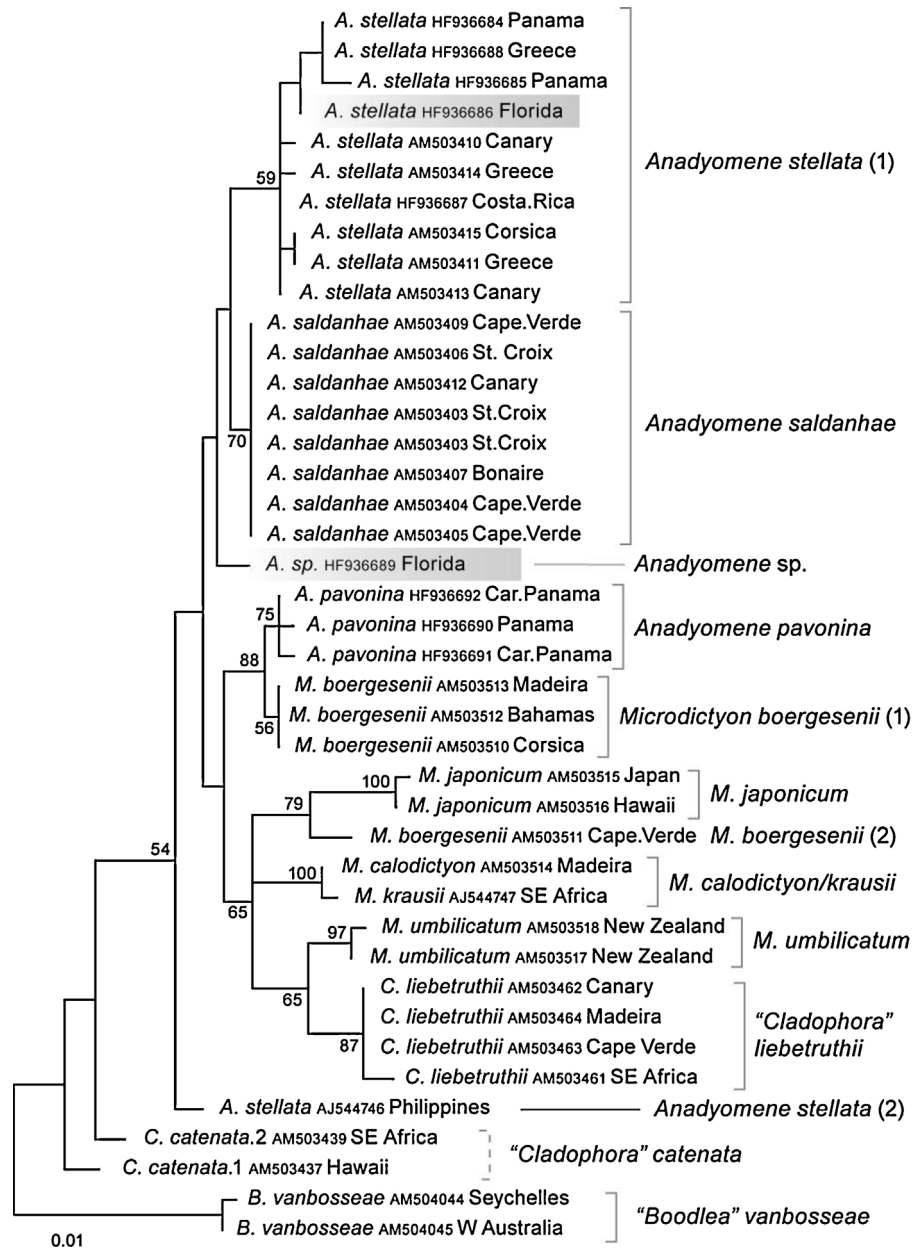


Fig. 3. Maximum likelihood tree of the *Anadyomene*/*Microdictyon* clade inferred from partial LSU nrDNA sequences, showing the phylogenetic position of *Anadyomene stellata* and *Anadyomene sp.* from Florida. ML bootstrap values (>50) are indicated at branches.

to canals had the highest variability, with four species showing significant variation in cover over the period between 2010 and 2012. The Central and Central Edge portions were apparently less dynamic with 2 species showing significant variation over time, and the South Edge and North Area were least dynamic, with only *Anadyomene sp.* showing significant variation over time.

4. Discussion

The persistent green macroalgal bloom in Biscayne Bay, Florida, is composed of two *Anadyomene* species. Thirteen *Anadyomene* species are described worldwide, from those, nine species are present in the tropical Western Atlantic coasts, 6 have an eperforated blade while only 3 have mesh-like thallus with perforated blade (Alves et al., 2011; Littler and Littler, 2012). The morphological identification of *A. stellata* from Biscayne Bay, confirmed based on available DNA sequences has an amphi-Atlantic distribution

with confirmed DNA records from the Mediterranean Sea (type locality), Canary Islands and the Caribbean Sea. Another specimen, previously identified as *A. stellata* from the Philippines (Leliaert et al., 2003), clearly belongs to a different species. *A. stellata* is closely related to *A. saldanhae*, which also has an amphi-Atlantic distribution, and *Anadyomene sp.* is sister to that clade. As in previously published LSU-based phylogenies, the monophyly of *Anadyomene* and *Microdictyon* was not supported (Leliaert et al., 2007a,b). With the provided morphological and molecular analysis, the identification of *A. stellata* is clear and robust. *A. stellata* has previously been reported for Florida (Littler and Littler, 2000; Dawes and Mathieson, 2008) and particularly to Biscayne Bay (Collado-Vides et al., 2011). However, this is the first report for this species reaching blooming characteristics.

*Anadyomene sp.* is a perforated species morphologically clearly separated from two perforated species reported for the Western Atlantic: *Anadyomene gigantiodictyon* Littler and Littler and

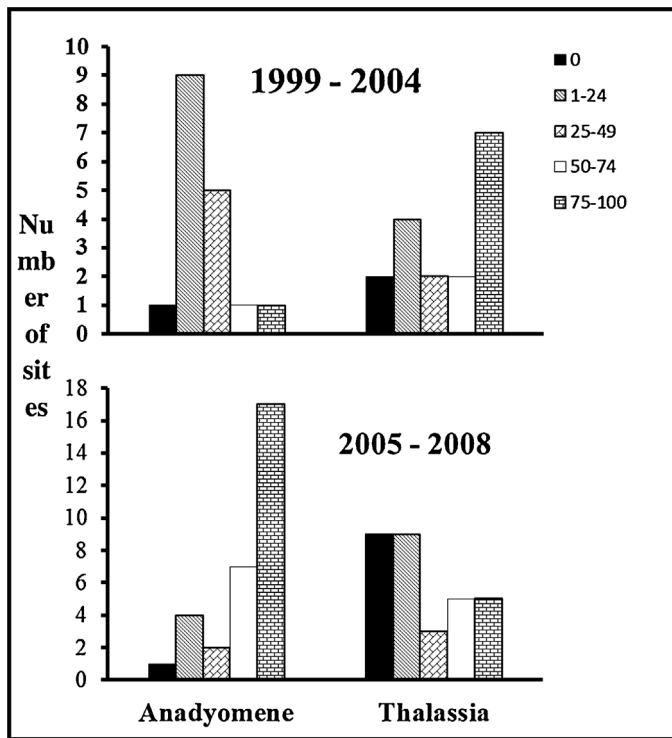


Fig. 4. Abundance of species on site revisited at different time intervals.

*A. pavonina*, (J. Agardh) Wille, the molecular data further confirmed that *Anadyomene* sp. is distinct from *A. pavonina*; at present DNA sequence data is lacking for the other perforate species, *A. gigantiodictyon*. The morphological characteristics, such as cell size and general cell and branching patterns of our specimens closely resemble the third eperforate described species for the Western Atlantic *Anadyomene linkiana* Littler and Littler a diminutive perforated species reported for deep waters of the Bahamas (Littler and Littler, 1991), however some distinct morphological characteristics were absent in our material. The original description of *A. linkiana* mentions the presence of diminutive stipe formed by 3–9 rhizoidal elongations. In our specimens, distinct stipes were lacking,

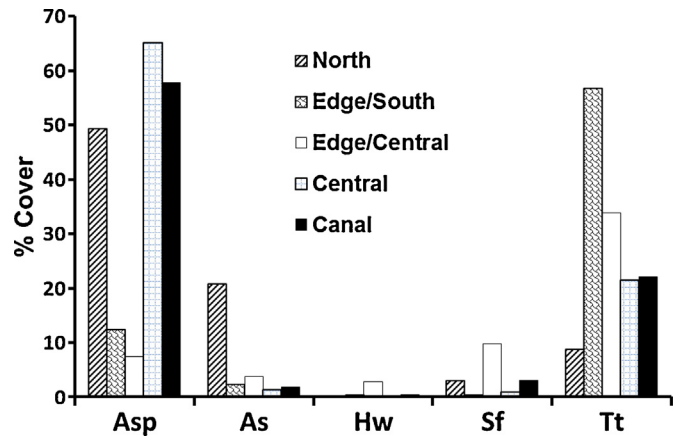


Fig. 5. Overall average % cover of species per area. Asp, *Anadyomene* sp.; As, *Anadyomene stellata*; Hw, *Halodule wrightii*; Sf, *Syringodium filiforme*; Tt, *Thalassia testudinum*.

although small rhizoids were detected. The absence of stipes can be attributed to the drifting habit acquired during blooming stage, it is well documented that drifting algae can modify some of their morphological characteristics when losing their attachment to substrate including the loss of their holdfast (Norton and Mathieson, 1983). A second difference is the absence of vein-encircled perforation described for mature specimens, which is likely a consequence of the fact that the bloom-forming specimens were drifting specimens in rapid blooming growth maintaining juvenile characteristics. These morphological inconsistencies and the lack of DNA data from the original *A. linkiana* do not allow us to completely identify our material with this taxon. Further investigations are needed to confirm the identity of *Anadyomene* sp. as *A. linkiana*; or as a new species, in either case it will be a new record for Florida.

Several cases have been reported in coastal ecosystems where nutrient enrichment can degrade an ecosystem functioning; examples are from freshwater and estuarine environments (Scheffer and van Nes, 2007; Frankovich et al., 2011) and seagrass beds (McGlathery, 2001; Williams, 2007). Increased nutrient loadings in coastal waters have been associated with a shift in dominance from seagrass and perennial macroalgae to ephemeral, bloom-forming

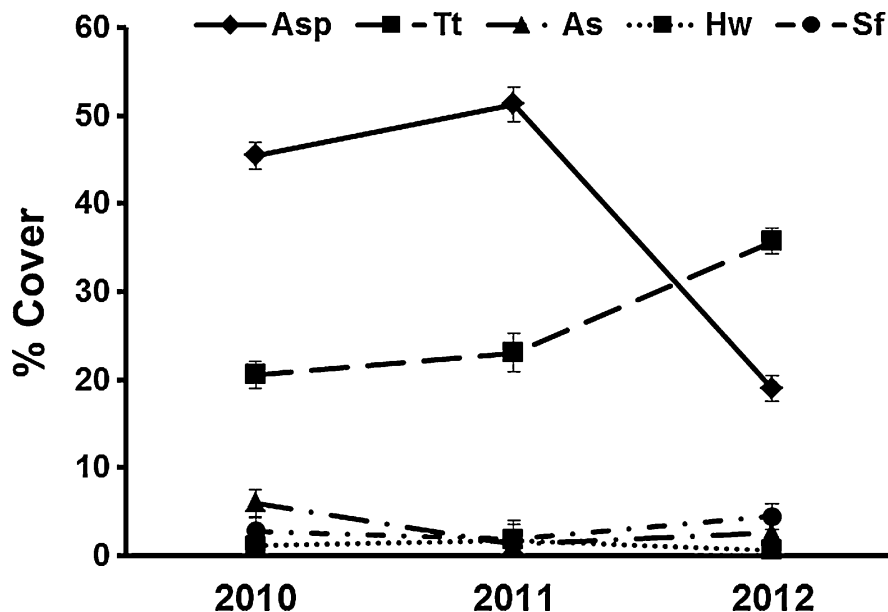


Fig. 6. Overall temporal abundance of species. Asp, *Anadyomene* sp.; As, *Anadyomene stellata*; Hw, *Halodule wrightii*; Sf, *Syringodium filiforme*; Tt, *Thalassia testudinum*.

**Table 2**

One-way ANOVA per area with year as factor. Asp, *Anadyomene* sp.; As, *Anadyomene stellata*; Hw, *Halodule wrightii*; Sf, *Syringodium filiforme*; Tt, *Thalassia testudinum*. Only significant differences are presented.

Species	Sum of squares	df	Mean square	F	p
Area 1: Close to canal					
Asp	41332.019	2	20666.010	14.603	<0.0001
As	196.884	2	98.442	4.065	0.018
Tt	25603.297	2	12801.648	18.736	<0.0001
Hw	39.022	2	19.511	3.666	0.027
Area 2: Central area of the bloom					
Asp	32526.717	2	16263.358	14.403	<0.0001
Tt	10309.844	2	5154.922	8.320	<0.0001
Area 3: Central edge					
Tt	9392.446	2	4696.223	4.980	0.008
Hw	1508.585	2	754.293	8.491	<0.0001
Area 4: South Edge					
Asp	3053.225	2	1526.613	3.164	0.049
Area 5: North area					
Asp	28273.691	2	14136.845	11.153	<0.0001

algae (Anderson, 2009; Ye et al., 2011), a problem also observed in coral reefs affected by ephemeral algal blooms (Smith et al., 2005). Nevertheless not all species develop into large blooms, the simple morphology of *Anadyomene* species with very high SA:V is similar to other green blooming macroalgae known to incorporate nutrients rapidly (Littler and Littler, 1980). For example *Ulva* Linnaeus in Finland (Blomster et al., 2002), *Cladophora glomerata* (Linnaeus) Kützing in the Baltic Sea (Gubelit and Berezina, 2010), *Cladophora sericea* (Hudson) Kützing in Denmark (Thybo-Christesen et al., 1993), all simple single cell layers, or uniseriate branching algae, have developed green algal blooms all around the coastal waters of the planet (see Ye et al., 2011 for a review).

Our extensive surveys indicate that, so far, the bloom of these two thin green macroalgal species is limited to the north-central, inshore areas of the bay. This distribution coincides with a region characterized by Briceño et al. (2011) as enriched by nutrients coming from canal discharges likely facilitating the rapid growth of fast growing algal species, as documented here where the highest abundance of *Anadyomene* sp. was found close to areas affected by canal discharges. Additionally the geology/hydrology of southeast Florida, and Biscayne Bay, has been documented to support significant groundwater discharge to the bay (Langevin, 2003). The part that groundwater discharge may play in the persistence of the bloom in

the central area needs to be further examined (Stalker et al., 2009). The temporal and spatial dynamics found showed that historically, the establishment of the bloom resulted in a detrimental effect on the seagrasses; furthermore the significant temporal and spatial differences across areas might reflect the dynamics of nutrient sources and hydrological characteristics of the area (Stalker et al., 2009).

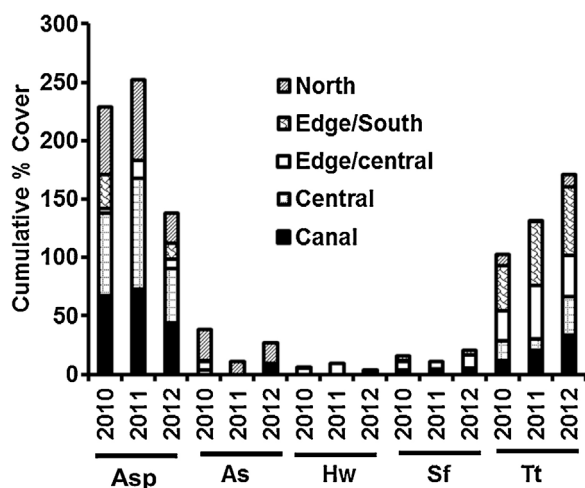
Biscayne Bay is a fragile ecosystem at the edge of a densely populated and highly developed area, as well as a large port supporting high volumes of freight shipping and passenger ships. The presence of a bloom of a morphological simple species, including a new record for the area, might be a symptom of nutrient enrichment that facilitates the expansion of seaweeds. The blooming species completely covered extensive areas of pre-existing seagrass bed, resulting in the loss of significant coverage of seagrasses. The impacts to the seagrass habitats in Biscayne Bay can have dramatic effects in the ecosystem as demonstrated in other shallow water seagrass environments (Williams, 2007; Nelson et al., 2008).

## 5. Conclusions

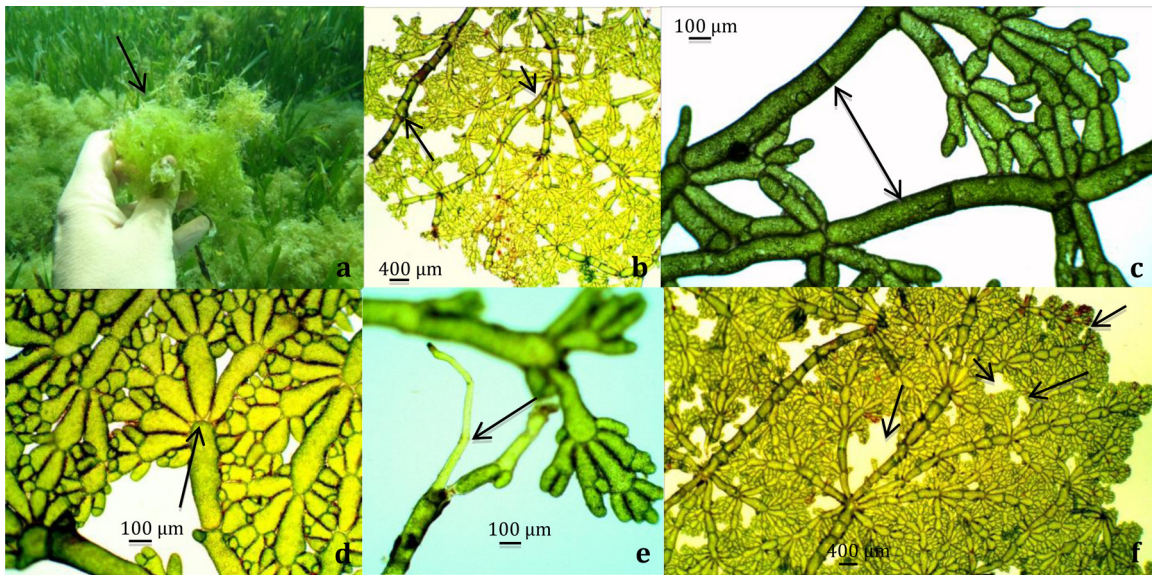
In this study we reported an *Anadyomene* species complex persistent bloom in Biscayne Bay, Florida, which might be an important sign of nutrient loadings in the Bay. This bloom is composed by *A. stellata*, and *Anadyomene* sp., two green macroalgae, adding two species to the list of green bloom forming macroalgae (Ye et al., 2011; Williams, 2007). In the case of *Anadyomene* sp. this report highlights the potential introduction of a new species blooming once is present in rich waters, a possible phenomenon that we might experience more frequently in coastal areas. The spatial and temporal distribution of the blooming species is related with areas affected by land nutrient sources such as canals, and possibly groundwater discharges.

## Acknowledgments

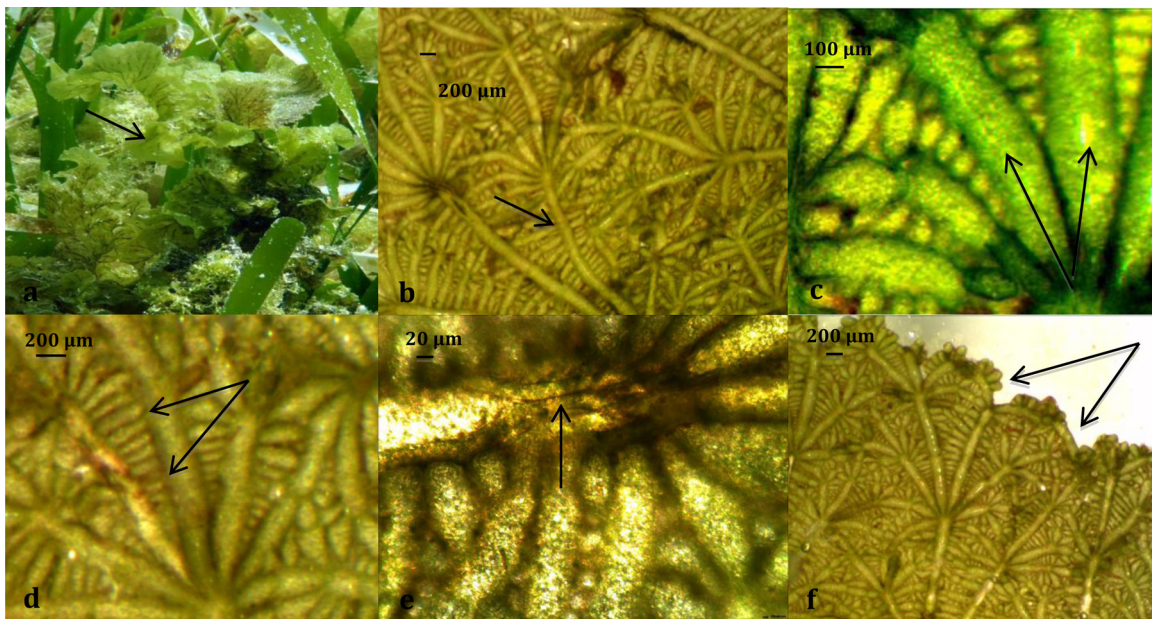
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**Fig. 7.** Cumulative abundance of species per area and year. Asp, *Anadyomene* sp.; As, *Anadyomene stellata*; Hw, *Halodule wrightii*; Sf, *Syringodium filiforme*; Tt, *Thalassia testudinum*.



**Fig. A.1.** *Anadyomene* sp. morphological characteristics. (a) Thallus habit; (b) view of deumbent perforated mesh, arrows sign uniseriate proximal veins; (c) detail of juvenile mesh at its proximal portion arrows show main uniseriate vein; (d) detail of proximal portion of adult mesh, arrow show polychotomous branching; (e) detail of early stages of the mesh arrow sign rhizoidal elongation; (f) distal portion of the mesh showing decreasing perforations, arrow sign anastomosis of marginal cells and decrease of perforation size.



**Fig. A.2.** *Anadyomene stellata* morphological characteristics. (a) Thallus habit; (b) view of eperforated blade, arrows sign uniseriate mid veins; (c) detail of proximal portion of blade, arrow show polychotomous branching and swollen cells at their distal portion; (d) mid portion of the blade, arrow sign parallele interstitial cells; (e) proximal section of the blade, arrow sign rhizoid detail; (f) distal section of the blade showing blade lobulal margin, arrows sign marginal oval cells.

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**Appendix A.**

See Figs. A.1 and A.2.

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