NOTES ON THE MARINE ALGAE OF THE INTERNATIONAL BIOSPHERE RESERVE SEAFLower, CARIBBEAN COLOMBIA IV: NEW RECORDS OF MACROALGAL EPIPHYTES ON THE SEAGRASS *THALASSIA TESTUDINUM*

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ABSTRACT

Nine species of macroalgae are newly reported for the Caribbean International Biosphere Reserve Seaflower. Of these taxa, *Neosiphonia sphaerocarpa*, *Polysiphonia schneideri*, *Polysiphonia sertularioides*, *Cladosiphon occidentalis*, and *Phaeophila dendroides*, have been previously reported from Colombian waters, whereas *Ulothrix* sp., *Ulva flexuosa* subsp. *paradoxa*, *Chaetomorpha minima*, and *Cladophora liniformis* represent new records for the country. All the algae were found growing epiphytically on *Thalassia testudinum* in shallow (<1 m) seagrass meadows around San Andrés Island. Their morphological features are discussed.

KEY WORDS: Colombia, Epiphyte, Marine algae, New records, *Polysiphonia*.

RESUMEN

Notas sobre las algas marinas de la Reserva Internacional de Biosfera Seaflower, Caribe colombiano IV: nuevos registros de macroalgas epífitas sobre hojas de *Thalassia testudinum*. Se registran por la primera vez para la Reserva Internacional de Biosfera Seaflower nueve especies de macroalgas. De estas especies, *Neosiphonia sphaerocarpa*, *Polysiphonia schneiderii*, *Polysiphonia sertularioides*, *Cladosiphon occidentalis* y *Phaeophila dendroides*, han sido registradas previamente para aguas colombianas, mientras *Ulothrix* sp., *Ulva flexuosa* subsp. *paradoxa*, *Chaetomorpha minima* y *Cladophora liniformis* son nuevos registros para el país. Todas las algas fueron encontradas epífitas sobre hojas de *Thalassia testudinum*, en praderas someras (<1 m) en la isla de San Andrés. Se discuten sus características morfológicas.

PALABRAS CLAVES: Colombia, Epífitos, Algas marinas, Nuevos registros, *Polysiphonia*.
INTRODUCTION

Seagrass meadows are very productive ecosystems of which a large proportion is often attributed to the epiphytes (Leliaert et al., 2001; Won et al., 2010). Epiphytes can represent up to 50% of the total above-sediment biomass of a seagrass meadow (Leliaert et al., 2001). Epiphytes can therefore play an important role in the functioning of seagrass ecosystems.

The most widely distributed seagrass in the Caribbean is *Thalassia testudinum* Banks ex König, which provides ample substrate for algal epiphytes (Cho et al., 2002; Barrios and Díaz, 2005; Corlett and Jones, 2007; Samper-Villarreal et al., 2008). However, few studies characterizing the epiphytic flora have been addressed in the Caribbean, there being only those in Florida (Dawes 1987, Won et al., 2010), in Costa Rica (Samper-Villareal et al., 2008), and in Venezuela (Barrios and Díaz, 2005). In Colombia, the studies on macroalgae epiphytes have been restricted to estimations of their biomass (Palacios et al., 1992). Our recent field surveys on *Thalassia testudinum* macroalgal epiphytes in San Andrés Island revealed some species previously unknown to the region (Albis-Salas and Gavio, 2011). We present nine new records for the Archipelago, three species of red algae, one brown alga and five green algae. Four taxa represent new records for Colombia. All these taxa are generally overlooked in the floristic treatments of the region, mainly due to their small size and difficult taxonomic treatment. We herein provide detailed morphological features of the specimens encountered and a comprehensive discussion on the taxonomic status of each species.

MATERIALS AND METHODS

San Andrés (12°28’55”N; 81°40’49”W) is an oceanic island situated in the southwestern Caribbean, Colombia (Figure 1), being part of the San Andrés and Old Providence Archipelago, declared as International Biosphere Reserve Seaflower since 2000 (Coralina, 2007). For details on the study site, see Albis-Salas and Gavio (2011). During the wet (December 2007) and dry seasons (March 2008) we sampled in six sites, on the east coast of the island (Gavio et al., 2010; Albis-Salas and Gavio, 2011). All meadows are shallow (<1 m). The leaves of *Thalassia* were preserved in a 4% formalin/seawater solution. In the laboratory, algae were observed under an Olympus BX 51 microscope and identified with specialized bibliography for species identification (Littler and Littler, 2000; Dawes and Mathieson, 2008; Littler et al., 2008; Stuercke and Freshwater, 2010). Portions of the thalli were mounted on glass slides in 50% glycerin, after staining in aniline-blue solution. Information on the type localities of these taxa has been obtained from Silva (2013).
Figure 1. San Andrés Island with study sites. 1. Punta Hansa (12°58'34.6" N -81°69'01.0" W), 2. Harbor (12°57'60.9" N -81°70'20.1" W), 3. Bahía Hooker (12°34'09.1" N -81°42'10.3" W), 4. Bahía Honda (12°33'70.7" N -81°42'41.9" W), 5. La Mansión (12°69'01.0" N - 81°70'39.3" W) and 6. Rocky Cay (12°54'35.8" N -81°70'40.9" W).
RESULTS AND DISCUSSION

We report a total of nine new records for San Andrés Island, four of which are new for Colombia. All species were found as epiphytes on leaves of *Thalassia testudinum*. We report three species of Rhodophyta, one Heterokontophyta and five Chlorophyta. Furthermore, this is the first report of the genera *Cladosiphon*, *Neosiphonia*, *Phaeophila* and *Polysiphonia* for the San Andrés and Old Providence Archipelago, and of the genus *Ulothrix* for Colombia.

The algae were mostly diminutive filamentous specimens growing as epiphytes or endophytes on the encrusting algae *Hydrolithon farinosum* and *Pneophyllum fragile* which were in turn overgrowing leaves of *Thalassia testudinum*. New records for Colombia are marked with an asterisk [*].

**RHODOPHYTA**

**Order Ceramiales**

**Family Rhodomelaceae**


**Type locality:** St. Thomas, Virgin Islands.

Thallus filamentous, bushy, maroon in color, up to 1 cm tall. Branching alternate to pseudodichotomous. Thallus attached by discoid holdfast and secondarily by unicellular rhizoids that arise from distal ends of ventral pericentral cells with cross wall. Branches 60-90 µm diam, with four pericentral cells per segment, segments 80-140 µm long, 0.5-1.5 diameters long. Tetrasporangia 10-12 µm wide, 20-25 µm long, tetrahedrally divided, in spiral series (Figure 2a). Spermatangial branchlets cylindrical and lateral, produced on lower segments of apical filaments (Figure 2b). Cystocarps oval, 183 µm wide, 178 µm long (Figure 2c).

**Site and season of collection:** Dry season 07-10/12/2007, Bahía Honda, Punta Hansa.

**Known western Atlantic distribution:** Barbados, Belice, Cuba, Florida, Hispaniola, Lesser Antilles, Puerto Rico, Venezuela, Virgin Islands.

*Polysiphonia schneideri* B. Stuercke and D.W. Freshwater (2010)

Reported by Díaz-Pulido and Díaz-Ruiz (2003) as *P. denudata*.

**Type locality:** Wrightsville Beach, North Carolina, USA.

Thallus filamentous, creeping, red-maroon to violet, without cortication (Figure 2d). The base was not observed in our specimens; however, there is a prostrate axis 130-180 µm diam, 0.8-1 diameters long, from which erect axes arise. Erect axes may reach 5 cm in length, frequently ramified, alternate proximally, unilateral
distally. Lateral branches thinner, 70-100 µm diam; segments 1.5-2 diameters long; branches abruptly tapering distally to 50-55 µm diam, 1-1.5 diameters long; toward the apex the tapering is stronger, to 30-40 µm diam, 0.5 diameters long. Lateral adventive branches present. Pericentral cells 6-7 (Figure 2e). Apex conspicuous, 10-12.5 µm long, 10-12 µm diam. The branches are borne axillary to the trichome (Figure 2f). Trichoblasts abundant towards the apex, 2-3 times dicho- to subdichotomously ramified. Scar cells frequent and irregularly arranged. Rhizoids digitiform, cut-off from ventral pericentral cells, abundant, generally one per segment but we sometimes observed two rhizoids per segment, 20-25 µm diam, 500-800 µm long. Tetrasporangia in series, ellipsoidal, in the middle part of the thicker axes, sometimes dispersed in the whole thallus, 30-50 µm diam, 90-100 µm long. Spermatangial branchlets narrowly ovate and lateral, produced on lower segments of apical filaments, 18.2-32 µm diam 92-124 µm long (Figure 2g).

Known western Atlantic distribution: Bermuda, Colombia, Florida, Panamá, Puerto Rico, Texas, Venezuela.

Site and season of collection: Dry season 29-30/03/2008, Punta Hansa, Rocky Cay.

Remarks: Stuercke and Freshwater (2010) recently determined that the western Atlantic taxon known as *Polysiphonia denudata* is a previously unrecognized species, which they named *P. schneideri*. In their study, they included vouchers from continental Colombia, which belong to this new identity. The specimen that we found agrees with their description of *P. schneideri*, with the exception of the position of the tetrasporangia. Those authors described the tetrasporangia as being disposed in the distal portion of the branches, while our specimens had the tetrasporangia in the middle portion of the largest axes. Furthermore, tetrasporangia in our specimens were slightly smaller (30-50 μm diam) than those reported by Stuercke and Freshwater (2010, 45-85 μm diam), as well as the spermatangial branches (18-32 μm diam x 92-124 μm long in our samples versus 35-60 μm diam x 125-260 μm long in the original description). Whether these variations represent normal variability in a population or reveal further cryptic species diversity should be assessed with molecular data.

*Polysiphonia cf. sertularioides* (Grateloup) J. Agardh (1863)

Type locality: Cette, Gulf of Lion, France.

Thallus filamentous, creeping, red to maroon, 0.5-5 mm tall. Erect branches alternate proximally, dichotomous distally. Prostrate axis (33) 75-100 μm diam, with four pericentral cells, segments 1-2 diameters long (Figure 3a). Erect axes (30) 40-55 μm diam, segments 0.5-3 diameters long. In some specimens secondary branching is rather sparse: the first branch may appear after 10-25 segments, and there may be 10-12 segments between branches; however, in adventitious short branches we observed an interval of only 2-4 segments between branches. Branchlets constricted at the base and gradually tapering toward the apex (Figure 3a), although we occasionally found specimens that were abruptly tapering toward the apex. Lateral branches forming in axils of trichoblasts. Trichoblasts deciduous, 1-3 times dichotomous to subdichotomous branched, with obvious scar cells spirally arranged. In some plants, we observed that scar cells appear after 5-9 segments. Scar cells give rise to adventitious branches. Rhizoids unicellular or multicellular, finger-like, cut off from parental cells (Figure 3b). Tetrasporangia spherical 70-90 μm, strongly spiraled in outer branchlets (Figure 3c). Spermatangial branchlets cylindrical on lower segments of trichoblasts (Figure 3d). Cystocarps oval, 130-190 μm wide, 240-270 μm long (Figure 3e).
Figures 3. a-e. *Polysiphonia cf. sertularioides*. a. Habit, adventitious branches. Scale bar = 60 μm. b. Rhizoids cut off from parental cells. Scale bar = 20 μm. c. Tetrasporangia spiraled in outer branchlets. Scale bar = 100 μm. d. Spermatangial branchlets on lower segments of trichoblasts (arrowhead). Scale bar = 100 μm. e. Cystocarp. Scale bar = 50 μm. f-h. *Cladosiphon occidentalis*. f. Habit. Scale bar = 2 mm. g. Terminal moniliform cortical filaments (cf) and a typical phaeophycean hair (h). Scale bar= 30 μm. h. Plurilocular (ps) and unilocular sporangia (us). Scale bar = 100 μm.

**Site and season of collection:** Wet season 07-10/12/2007, Harbor, Punta Hansa; dry season 29-30/03/2008, Harbor, Punta Hansa.
**Known western Atlantic distribution:** Bahamas, Belize, Colombia, Cuba, Florida, Panamá, Texas, Venezuela.

**Remarks:** The specimens we observed presented great morphological variation in thallus size and branching pattern. We observed individuals with scattered branching and prostrate axis of 33-40 µm diameter, and others with frequent branching and prostrate axis of 75-100 µm in diameter. There was scar cells variation as well, with specimens showing spirally arranged scar cells every segment, while others with scar cells appearing after the first 5-9 segments of the branch. Furthermore, sometimes the filaments gradually tapered towards the apex, while in other plants the tapering was rather abrupt.

Womersley (1979) proposed that *P. sertularioides*, originally described from the Mediterranean Sea, and *P. flaccidissima* Hollenberg, described from the Pacific coast of North America and later reported for the tropical Pacific, Caribbean Sea, and South Africa (Rojas-González and Afonso-Carrillo, 2010), should be considered taxonomic synonyms because they share many diagnostic characters, such as prostrate habit, rhizoids with close connection, conspicuous trichoblasts, presence of adventitious branches, frequent scar cells, lateral branches forming in axils of trichoblasts and spirally arranged trichoblasts. Later, Kapraun et al. (1983), Abbott (1999) and Womersley (2003) again suggested synonymy, pending new research. Abbott (1999), however, pointed out that *P. flaccidissima* has a much more developed prostrate system than *P. sertularioides*. The description of *P. sertularioides* by Lauret (1967), in his extensive work on the Mediterranean *Polysiphonia*, is very similar to Hollenberg (1942) original description of *P. flaccidissima*. However, there are differences between the two taxa which have been later dismissed by other authors. According to Lauret (1967), there is a very clear pattern in scar cell distribution in specimens of genuine *P. sertularioides*: in the upright segments there is always a scar cell before a branch, and after it there is a segment without scar cell, with a pattern SBN (scar cell, branch, no scar cell). On the other hand, in the original plates of Hollenberg (1942, p. 775, fig. 8) for *P. flaccidissima*, the pattern is reversed, i.e. the branch is preceded by a segment without scar cell and followed by one with scar cell (NBS). The robustness of this pattern as a taxonomic feature has not been considered again by other authors. In our specimens we mostly found a SBS pattern (scar cell, branch, scar cell), but in some specimens we observed also a SBN pattern, as in Hollenberg’s original description. As we already mentioned, the morphological variation that we observed among specimens fitting the description of *P. sertularioides* was rather high, and this character was polymorphic as well. Mamoozadeh and Freshwater (2011), in a recent molecular study on Caribbean *Polysiphonia*, found genetic variation among three specimens of *P. cf. sertularioides*
from Panama, indicating that the taxon is possibly a species-complex of cryptic taxa. Since the most recent published works on *Polysiphonia* maintain the synonymy between *P. flaccidissima* and *P. sertularioides*, we decided to follow this trend. As many other authors suggested, a thorough revision of the *Polysiphonia sertularioides/flaccidissima* complex is needed. Díaz-Pulido and Díaz-Ruiz (2003) reported *Polysiphonia flaccidissima* for the continental coast of Colombia in the Caribbean.

**HETEROKONTOPHYTA**

**Order Ectocarpales**

**Family Chordariaceae**

*Cladosiphon occidentalis* Kylin (1940)

**Type locality:** Dry Tortugas, Florida, USA.

Thalli erect, light brown to olive brown in color, soft and mucous, up to 11.5 cm high, with a monostromatic discoid holdfast, 0.5-1 mm diam, from which a main axis arises. Axes cylindrical (Figure 3f), to 1.5 mm in diameter, branched with unilateral to irregular branches abundant at base and sparse toward the apex, with short second-order branches up to 1 mm diam (Figure 3f). Medulla multiaxial, consisting of longitudinal filaments, the axis becoming hollow a short distance behind its apex. Branches often ending in a hair laterally displacing the distal portion of the medullary filaments (Figure 3g). Medullary cells 110-225 µm long and 30-75 µm diam. Thin subcortex 1 cell thick formed perpendicularly to the medullary filaments. Subcortical cells hyaline, subcylindrical to broad at base, (5)10-15 µm diam (Figure 3h). Primary cortical filaments simple, 100-225 µm long, composed of 6-13 cells, with proximal cells cylindrical, 17.5-27.5 µm long, 5-7.5 µm diam. Distal cells moniliform, 7.5–12.5 µm long, 7.5–12.5 µm diam (Fig. 3g). Phaeophycean hairs abundant, arising from subcortical cells, with a short, basal sheath 11-12.5 µm diam (Figure 3g).

Plurilocular sporangia in groups of 3-6, on distal cells of cortical filaments, 25–26 µm long and 10-12.5 µm diam. Unilocular sporangia ovoid, 25-50 µm long and 25–45 µm diam, sessile and solitary, borne on proximal cells of cortical filaments or on distal subcortical cells (Fig. 3h).

**Site and season of collection:** Wet season 07-10/12/2007, Harbor; dry season 29-30/03/2008, Harbor.

**Known western Atlantic distribution:** Bahamas, Belize, Cuba, Florida, Panamá, Texas, Virgin Islands.
CHLOROPHYTA
Order Ulotrichales
Family Ulotrichaceae
*Ulothrix* sp.

Thallus minute, pale green, formed by simple uniseriate filaments to 1.1 cm high (Figure 4a). Filaments straight, neither upcurved nor bent. Cells cylindrical, 16 µm diam, 15-18 µm long with smooth cell walls to 3 µm thick. One chloroplast per cell, conspicuous, H-shaped, with one pyrenoid (Figure 4b). Apical cell rounded at tip, 10 µm, 12 µm long.

Figures 4. a-b. *Ulothrix* sp. a. Habit. Scale bar = 100 µm. b. Detail of single chloroplast per cell. Scale bar = 10 µm. c. *Ulva flexuosa* subsp. *paradoxa*. Habit and detail of cells. Scale bar = 100 µm. d. *Phaeophila dendroides*. Habit with hairs (h) grow out from cells. Scale bar = 50 µm. e. *Chaetomorpha minima*. Habit. Scale bar = 100 µm. f. *Cladophora liniformis*. Habit. Scale bar = 500 µm. g. Detail of branch per node. Scale bar = 100 µm.
Site and season of collection: Dry season 29-30/03/2008, Harbor.

Remarks: Wynne (2011) included two species of Ulothrix in his checklist of benthic algae of the tropical and subtropical Western Atlantic, Ulothrix flacca and U. subflaccida. These two species are distinguished by the basal portion, attached by a basal cell and rhizoids formed as down-growing extensions from a few intercalary cells above in U. flacca and by tapering rhizoidal basal cells in U. subflaccida (John, 2007). In the single specimen we found, it was not possible to observe the basal portion of the plant, which is an important character to distinguish among the Ulothrix species present in the Caribbean flora. However, we consider this alga to be a member of the genus Ulothrix because of its diminutive habit, and the presence of just one chloroplast per cell (the genus Chaetomorpha has several, with the exception of C. philippinensis, Leliaert et al., 2011) (Figure 4b). The genus Uronema is composed of diminutive species, mostly restricted to freshwater habitat. The only marine species reported to date, Uronema marinum, is much smaller in size, the longest filament size to 600 µm, according to Kraft (2007), the chloroplast has a different appearance, and the apical cell is larger than the other cells (see Figure 6, p. 22 in Kraft, 2007). This is the first record of the genus for Colombia.

Order Ulvales
Family Ulvaceae
*Ulva flexuosa subsp. paradoxa* (C. Agardh) M.J. Wynne (2005)

Type locality: Bangor, Wales.

Thallus flaccid, to 3 cm high, light green, branching abundant below, opposite to irregular, 30-325 µm diam (Figure 4c). Holdfast conspicuous. Main axis to 16 cells randomly arranged. Branchlets 1-4 cells thick. Cells rectangular to polygonal, 5-12.5 µm diam. and 5-15 µm long (Figure 4c).

Site and season of collection: Wet season 07-10/12/2007, Bahía Hooker, Harbor, Rocky Cay; dry season 29-30/03/2008, Bahía Hooker, Harbor

Known western Atlantic distribution: Bahamas, Barbados, Brazil, Cuba, Curaçao, Florida, Hispaniola, Jamaica, Lesser Antilles, Panamá, Puerto Rico, Texas, Venezuela, Virgin Islands.

Family Phaeophilaceae

*Phaeophila dendroides* (P.L. Crouan and H.M. Crouan) Batters (1902)

Type locality: Brest, Finistère, France.

Thallus of uniseriate branched filaments, endophytic in Hydrolithon farinosum and Pneophyllum fragile. Cells cylindrical, 4.5-5 µm diam, 17.5-27.5 µm long with many irregular swellings (Figure 4d). Hairs grow out from vegetative cells, are undulate without cross-walls at base (Figure 4d).
Site and season of collection: Wet season 07-10/12/2007, Harbor, Punta Hansa, Rocky Cay; dry season 29-30/03/2008, Bahía Honda, Bahía Hooker, Harbor, La Mansión, Punta Hansa, Rocky Cay.

Known Caribbean distribution: Florida, Hispaniola, Panamá, Texas, Venezuela, Virgin Islands.

Order Cladophorales
Family Cladophoraceae
*Chaetomorpha minima* Collins and Hervey (1917)

Type locality: Bermuda.

Thallus filamentous, inconspicuous, to 3 mm high, yellow-green. Filaments unbranched, uniseriate (Figure 4e), cells cylindrical, 7.5-20 µm diam and 37.5-100 µm long, 2-3 diameters long (Figure 4e), cells longer toward the base of the filaments. Apical cell blunt, 10-15 µm diam and 50 µm long. Attached by disc-like or finger like pad.

Site and season of collection: Wet season 07-10/12/2007, Bahía Honda, Bahía Hooker, Harbor, La Mansión, Punta Hansa, Rocky Cay; dry season 29-30/03/2008, Bahía Honda, Bahía Hooker, Harbor, La Mansión, Punta Hansa, Rocky Cay.

Known Caribbean distribution: Bermuda, Cuba, Florida and Venezuela.

Remarks: Although very common as individual filaments, it was not observed to form mats on the host plant as reported by Littler *et al.* (2008) in the Indian River Lagoon, Florida. It can attach either directly to *Thalassia* leaves or its epiphytic coralline algae (*Hydrolithon farinosum* and *Pneophyllum fragile*).

*Cladophora liniformis* Kützing (1849)

Type locality: Lagoon of Venice (Chioggia), Italy.

Thallus bright yellow-green in older parts and dark green in younger cells. Thalli forming indefinite masses floating at the surface of protected waters among seagrass beds, loose-lying on protected sediments bottoms or like small specimens to 2.5 mm high above seagrass blades. Plants having an irregular organization alternate to pseudodichotomously below and unilateral above, with irregular scattered branch and branches with different lengths (Figure 4f). Growth by division of apical and intercalary cells followed by cell elongation. Branches predominately apically inserted, but subterminal insertion was also observed. One to three branches per node (Figure 4g). Ramification angle 40-80°. Chloroplasts rounded and may form a network. Apical cells mostly long and cylindrical, the end widened or slightly tapering, 15-20 µm diam, 180-250 µm long, 15-17.5 diameters long. Ultimate
branches 20-35 μm diam., 200-375 μm long, 12-12.5 diameters long. Main axes 20-25 μm diam., 350 μm long, 14-17.5 diameters long. Basal cells 35 μm diam., 350 μm long. Filaments thicken slightly towards the base, which may reach 35 μm diam. Cell wall thickness in ultimate branches less than 5 μm.

**Site and season of collection:** Wet season 07-10/12/2007, Bahía Honda; dry season 29-30/03/2008, Bahía Honda.

**Known Caribbean distribution:** Bahamas, Cuba, Curaçao, Jamaica, Lesser Antilles

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