Taxonomy and distribution of the green algal genus *Halimeda* (Bryopsidales, Chlorophyta) in Brazil

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ABSTRACT – (Taxonomy and distribution of the green algal genus *Halimeda* (Bryopsidales, Chlorophyta) in Brazil). *Halimeda* is a genus of calcified coenocytic green algae with a well known ecological importance in some tropical areas. Bleached calcified segments of *Halimeda* may accumulate in large deposits of economic potential as is the case in the northeastern coast of Brazil. In a survey of the genus in Brazil based on recent collections and examination of abundant material deposited on Brazilian herbaria we identified seven species: *Halimeda cuneata* Hering, *H. discoidea* Decaisne, *H. gracilis* Harvey ex J. Agardh, *H. incrassata* (Ellis) Lamouroux, *H. opuntia* (Linnaeus) Lamouroux, *H. simulans* Howe and *H. tuna* (Ellis & Solander) Lamouroux. These species are described in detail, with emphasis on diagnostic characters. Our study has shown that the shape and size of the utricula in surface view, under scanning electron microscopy, can be utilized to discriminate some species. Fertile specimens of *Halimeda cuneata* and *H. discoidea* are reported for the first time in the region. Data on vertical and geographical distribution are presented for each species and the southern limit of the genus in the western Atlantic was extended.

Key words - Brazil, Bryopsidales, *Halimeda*


Palavras-chave - Brasil, Bryopsidales, *Halimeda, Halimeda cuneata*

Introduction

The genus *Halimeda* Lamouroux is characterized by a coenocytic thallus constructed of a system of interwoven bifurcated siphonous filaments that expand into utricles at the thallus surface. Macroscopically, the thallus is characterized by a series of green articulated coin shaped segments made rigid by the impregnation of calcium carbonate as aragonite (Gilmartin 1960, Borowitska & Larkum 1977).

Species recognition has been based mainly on the gross morphology of the segments, branching pattern, position and shape of holdfasts. Anatomy provides additional distinctive characters such as the size and shape of the utricles and structure of the node joining two successive segments (Barton 1901, Taylor 1950, Hillis 1959, Hillis-Colinvaux 1980). Hillis-Colinvaux (1980) divided the genus into five sections based on the patterns of nodal structure: *Rhipsalis, Opuntia, Halimeda, Micronesicae* and *Crypticae*. Hillis et al. (1998) based on a partial sequence of the 18S rDNA found a good correlation between several morphological characters and the molecular data, but recognized only three lineages: *Rhipsalis, Opuntia* and *Halimeda*. More recently Kooistra et al. (2002) extended considerably the sequencing study of Hillis et al. (1998) including SSU and ITS 1 and 2 regions; although they recognized the relevance of nodullary siphons structure and
arrangement, they rejected the sectional treatment of Hillis-Colinvaux (1980).

The genus is broadly distributed in the tropics, being represented by 33 species (Hillis et al. 1998). The following species have been reported from Brazil: *Halimeda discoidea* Decaisne, *H. gracilis* Harvey ex J. Agardh, *H. incrassata* (Ellis) Lamouroux, *H. opuntia* (Linnaeus) Lamouroux, *H. simulans* Howe and *H. tuna* (Ellis & Solander) Lamouroux (Horta & Oliveira 2002). However their circumscription is not always clear and varies with the author.

This study is a critical revision of the species that occur in Brazil based on the analysis of abundant material. Information on scanning electron microscopy (SEM) of thallus surface is presented for the first time and a map showing the geographical distribution of the species along the Brazilian coast is provided, extending the southern limit of the genus in the American Atlantic.

**Material and methods**

Recent material was collected from the intertidal and infralittoral zones during low tide by skin diving from 1997 to 2000 on several places of the northeastern coast of Brazil, and dredged during an extensive national program to survey the Brazilian platform (REVIZEE). We also studied material collections deposited in Brazilian herbaria (ALCB, IPA, JPB, PEUFR, RFA, SP, SPF and UFP) and the lectotype of *Halimeda cuneata* at BM. For the sake of brevity only a few selected specimens of the long list of material studied are included here.

For light microscopy, specimens were preserved in 4% Formalin and decalcified in 20% HCl. Dried herbarium specimens were immersed in 50% glycerol for 24 hours and then processed as described for liquid preserved specimens. Nodal regions were dissected with needles, and the preparations were mounted in a 50% glycerol solution. Structure sizes are reported as the mean of ten measurements, with the extreme values indicated between brackets.

For scanning electron microscopy (SEM), small portions of mature segments were pre-fixed in the field in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer, pH 7.0, and kept on ice. The material was then rinsed in the same buffer, post-fixed in 2% osmium tetroxide, dehydrated in an ethanol series (30% to 100%), dried in hexamethyleldazilane (HMDS), sputter-coated with gold and observed in a MEV Phillips XL30.

**Results**

Although aware that the sections established by Hillis-Colinvaux (1980) are not supported by molecular data according to Kooistra et al. (2002) we decided to keep this traditional arrangement for the time being.

**Section Rhipsalis** J. Agardh ex De Toni

Characterized by having nodal medullary siphons fused all together for a short extension and with a sequence of pits. This section is represented in Brazil by *Halimeda incrassata* and *H. simulans*, both included in “lineage 1” of Kooistra et al. (2002).


Type locality: Jamaica

Figures 1, 20, 24, 25.

Thallus erect, single or gregarious, strongly calcified at the base and less so at the upper segments; color light-green, becoming whitish on drying; up to 27 cm long; branching mainly di- to tetrachotomous; holdfast bulbous; the first two basal segments cylindrical and the third one usually flabellate, about 10 mm wide and 8 mm long; upper segments subcylindrical, cuneate or reniform, 9 mm wide by 6 mm long (figure 1). Cortex with up to five layers of utricles; external utricles hexagonal in outline, 72 (52-92) µm in diameter in surface view (figure 20) and 81 (59-103) µm long in cross-section holding together even after decalcification (figure 24); every secondary utricle 53 (44-74) µm long, gives rise to two primary ones; nodal medullary siphons interwoven and fused all together for not more than 100 µm, with conspicuous pores (figure 25).


This species was found from Ceará through the state of Bahia (figure 42), collected from the intertidal up to a depth of 53 m. Populations from Pernambuco were found partially buried in sand among beds of the angiosperm *Halodule wrightii* Ascherson. Specimens...
from the subtidal are more robust and with the typical reniform segments, whereas the intertidal ones have subcylindrical segments.


Type locality: Puerto Rico

Figures 2, 22, 26, 27.

Thallus erect, single or gregarious, lightly calcified at the base and less so at the upper segments; color dark-green when alive, becoming light-green on drying; up to 14 cm long; branching mainly di-trichotomous; holdfast bulbous; the first two basal segments short, subcylindrical, subcuneate; upper segments discoid to reniform, with imbricated aspect and up to 12 mm wide and 9 mm long (figure 2). Cortex with up to four layers of utricles; external utricles keep together even after decalcification (figure 26), hexagonal in outline, and similar to *H. incrassata*, in surface view, but smaller with 38 (18-48) µm in diameter (figure 22) and 34 (22-48) µm long in cross section. Every secondary utricle supports 2-4 primary ones; secondary utricles 62 (55-92) µm long. Medullary siphons interwoven, with short nodal fusions, forming a single group with inconspicuous pores (figure 27).


This species was found in the state of Pernambuco, Fernando de Noronha Archipelago and the state of Bahia (figure 42), being collected from the intertidal up to a depth of 63 m. In Bahia this species occurs in dense intertidal populations among *Penicillus capitatus* Lamouroux, *Caulerpa* spp., *Udotea* spp. and *Halimeda opuntia*. Intertidal specimens are more vigorous and calcified than the subtidal ones. Williams & Blomquist (1947) were the first to report the occurrence of *H. simulans* in Brazil (coast of Pernambuco and Fernando de Noronha Archipelago), which was considered as uncertain by Oliveira Filho (1977), in the absence of material for confirmation. Later on, Ugadim & Pereira (1978) found it among material dredged from Pernambuco, which is now confirmed.

Section **Opuntia** J. Agardh ex De Toni

This section is characterized by having nodal medullary siphons fused in pairs for a short distance. It is locally represented by only one species, *Halimeda opuntia*, which is included in “lineage 5” of Kooistra et al. (2002).


Type locality: Jamaica

Figures 3-5, 21, 28-30.

Thallus erect, forming compact or loosely prostrate tufts, strongly calcified; color light-green to whitish up to 34 cm long; branching polystichous, attached to the substrate by multiple holdfasts; segments variable in shape, usually trilobate, flattened to cylindrical, sometimes twisted, auriculated to crenulated ca. 18 mm in diameter and 9 mm long; remaining segments discoid to cuneate, with up to 12 mm wide and 9 mm long (figures 3-5). Cortex with 3-5 layers of utricles dichotomously branched (figure 28); external utricles rounded to polygonal, lightly coalescent after decalcification, 29 (15-44) µm in diameter, in surface view (figure 21), and 30 (26-41) µm long in cross section. Every secondary utricle supports 2 primary ones. Nodal medullary siphons interwoven, fused in pairs, seldom in groups of 3-4, with short nodal fusions (figures 29, 30).


This is the most common and widespread species of *Halimeda* worldwide, being considered pantropical. In Brazil the it is more common in the intertidal, but was also dredged at 44 m, from Ceará State to Rio de Janeiro (figure 42). Its morphology is variable: on reef flats tufts are more compact and imbricated with twisted segments, whereas on reef vertical walls tufts are more loose, branching tend to become distichous and segments are auriculate assuming the cordate form described by Barton (1901).

This species may have a key ecological role due to its high biomass and percentage of substrate cover. In some places it forms dense beds inhabited by a large diversity of invertebrates; in others their dead segments accumulate in large amounts and are sporadically utilized as a source of carbonates.

Section *Halimeda*

This section is characterized by short nodal medullary siphons extensively fused in groups of 2-3. The following species are present in Brazil: *Halimeda cuneata*, *H. discoidea*, *H. tana* and *H. gracilis*. The first three are included in the “lineage 3” and the last one in “lineage 4” of Kooistra et al. (2002).


Type: SOUTH AFRICA: Natal: Durham, Krauss s.n., 1839 (Lectotype BM!, designated by Hillis-Colinvaux 1980)

Figures 11-17, 31-34.

Thallus erect, single, lightly calcified; color dark-green, becoming white or yellowish on drying; up to 25 cm long; branching sparse to dense, usually dichotomous but also trichotomous to irregular in places and with up to 8 segments on a series without furcation (figures 11-14); holdfast basal and small; the first two basal segments are cylindrical or subcylindrical; upper segments are flat, cuneate to trapezoid, sometimes discoid with a smooth and shiny surface measuring up to 25 mm wide by 20 mm long; most of the segments are joined by a sort of short stipe, 1 mm long and 6 mm in diameter (figure 15), composed by densely interwoven, torulose and thick-walled filaments (figure 16). Cortex with three layers of utricles, seldom four; external utricles goblet shaped, keeping firmly together after decalcification; fused utricles were seen occasionally; external utricles are polygonal in surface view (figure 17) with 52 (22-66) µm in diameter; in cross section they measure 104 (74-144) µm long; secondary utricles suport 2-4 primary ones (figure 31); nodal medullary siphons interwoven and fused in groups of 2-3 for a variable extent (figures 32, 33); above the node the medullary filaments have thick walls and are pigmented, and may have a small tuft of short utricles above the point of filament fusions (figure 32). Gametophores are produced from the primary utricles and have 4-7 pedunculated gametangia, 370 µm long and 140 µm in diameter (figure 34).


Additional material studied: SOUTH AFRICA:

Type locality: “Kamtschatka” [true provenance unknown].

Figures 6, 7, 18, 35-37.

Thallus erect, single, lightly to moderately calcified; color light-green to green yellowish becoming whitish on drying; up to 11 cm long; branching sparse to dense, di-trichotomous; holdfast single and small; segments globose to pear-shaped about 103 µm wide. Thallus prostrate, moderate to strongly calcified; color light-green to green yellowish becoming whitish on drying; up to 24 cm long; branching di-trichotomous; attachment by multiple holdfasts; segments subcylindrical, cuneate to reniform, with a smooth and shiny surface, about 46 mm wide and 25 mm long (figures 6, 7). Cortex with 2-3 layers of utricles 65 (74-92) µm long in cross-section (figure 35) and 57 (37-77) µm in diameter in surface view, often laterally fused in pairs (figure 18), holding together strongly even after decalcification; secondary utricles subglobose, 121 (92-210) µm wide; nodal medullary siphons interwoven and fused in groups of 2-3 for a short distance (figure 36). Gametophores branched, produced from secondary utricles on the surface or margin of the fertile segments, bearing 5-8 globose to pear-shaped gametangia about 103 µm wide and 159 µm long (figure 37).


General morphology present some variation on populations of different depth; intertidal specimens tend to be more calcified and densely branched, with smaller and thicker segments. This species was found from Ceará state through Espírito Santo State (figure 42) from the intertidal to 166 m depth. It was found in the State of Sergipe for the first time. Coloration is darker on specimens from the intertidal than from the subtidal specimens. This species often occurs together with *H. opuntia*. Fertile material was found only once, in November, 1997, in deep water.

**Halimeda gracilis** Harvey ex J. Agardh, Acta Universitets Lund 23:82. 1887.

Type locality: Sri Lanka.

Figures 8, 19, 38-40.

Thallus prostrate, moderate to strongly calcified; color whitish, becoming grayish on drying; up to 24 cm long; branching di-trichotomous; attachment by multiple holdfasts; segments subcylindrical, cuneate to reniform, with a smooth and shiny surface, margin undulate, about 18 mm wide and 11 mm long (figure 8); segments friable before decalcification, with utricles lightly coalescent after decalcification; cortex with two, rarely three layers of utricles, rounded in outline, 52 (44-67) µm in diameter in cross section. Every secondary utricle supports up to 8 primary ones; secondary utricles clavate (figure 38), 74 (56-92) µm wide and 170 (166-333) µm long. Medullary siphons interwoven, with extensive paired nodal fusions, eventually with three filaments fused together (figures 39, 40).

Selected material: BRASIL: CEARÁ: 01°43′S, 37°07′W, 54 m, 6-VI-1998, REVIZEE (PEUF35112); 03°50′S, 37°37′W, 166 m, 12-X-1995, REVIZEE (PEUF35095).
The genus *Halimeda* in Brazil


Type locality: Mediterranean Sea

Figures 9, 10, 23, 41.

Thallus erect, single, moderate to lightly calcified; color light to dark-green, up to 10 cm long; branching sparse to dense, di-trichotomous; holdfast single and small; basal segments strongly calcified, subcuneate to subcylindrical, remaining segments discoid to subcuneate, with a rough surface, up to 20 mm wide and 12 mm long (figures 9, 10). Cortex with usually three, but up to four layers of utricles (figure 41); external utricles keep lightly coalescent after decalcification; polygonal in outline in surface view, with cell walls thicker than the other species (figure 23); 44 (29-55) \(\mu\)m in diameter, in surface view, and 108 (59-196) \(\mu\)m long in cross section; every secondary utricle supports 6 or more primary ones; secondary utricles 48 (44-52) \(\mu\)m wide and 111 (85-177) \(\mu\)m long. Medullary siphons interwoven, with extensive or short nodal fusions in groups of 2-3 as in *H. cuneata* and *H. discoidea* (figures 32, 36).


Figure 42. Geographic distribution of *Halimeda* species on the Brazilian coast: \(\bigcirc\) = *Halimeda cuneata*, \(\bullet\) = *H. discoidea*, \(\square\) = *H. gracilis*, \(\blacksquare\) = *H. incrassata*, \(\triangle\) = *H. opuntia*, \(\Delta\) = *H. simulans*, \(\bigcirc\) = *H. tuna*. 

Additional material studied: SRI LANKA: W.H. Harvey 72 (Isotype and photo at BM)
This species was found from the state of Maranhão through the State Rio of Janeiro (figure 42) from the intertidal to 60 m depth. Intertidal populations are more calcified and with a smaller holdfast, with thicker, smaller and rougher segments, usually dark-green to brownish.

Key for the identification of *Halimeda* species reported from Brazil

1. With a bulbous holdfast; nodal medullary siphons fused in a single group (section *Rhipsalis*)
   2. Basal segments usually cylindrical, but also subcuneate or flabellate; primary utricles measuring over 50 µm in diameter ............................................................................................................. *H. incrassata*
   2. Basal segments subcuneate or reniform, imbricate; primary utricles measuring less than 50 µm in diameter ............................................................................................................. *H. simulans*

1. With one or several non-bulbous holdfasts; nodal medullary siphons fused in groups of 2-3.
   3. Attachment by several holdfasts; nodal medullary siphons fused in pairs for a short extension (not more than 100 µm) (section *Opuntia*) ............................................................................................................. *H. opuntia*
   3. Attachment by single or multiple holdfasts; nodal medullary siphons fused in groups of 2-3 for more than 100 µm (section *Halimeda*)
   4. Thallus flaccid and prostrate, attached by multiple holfasts; secondary utricles clavate .......... *H. gracilis*
   4. Thallus erect with a single conspicuous holdfast; secondary utricles not clavate
   5. Segments discoid; secondary utricles inflated, sub-globular .................................................. *H. discoidea*
   5. Segments sub-cylindrical, cuneate to reniform; secondary utricles not inflated
   6. Basal segments cylindrical, stipe-like; nodal region pedunculate or cushion-like .............. *H. cuneata*
   6. Basal segments subcuneate; nodal region sessile ..................................................................... *H. tuna*

Discussion

One of the problems in identifying species of *Halimeda* is that gross morphology varies among populations of the same species growing in different habitats, making this criterium unreliable as was already remarked by Barton (1901) and by subsequent authors (e.g. Hillis 1959, Stark *et al.* 1969, Hillis-Colinvaux 1980). This was fully supported by our observations based on a large sample of specimens from a broad range of depths. This plasticity is certainly the explanation for the confusions we found in local literature (e.g. Pereira 1974, Dantas 1994) and in herbaria. Identification at species level is possible only by a combination of several criteria including habitat, gross morphology and anatomy of mature segments and nodes. Particularly useful are the patterns of nodal medullary siphons fusion, in addition to the size, shape and distribution of the utricles as remarked long ago (Barton 1901). Nodal structure seems to vary less and, therefore, is more useful at the section level. Gametangia arrangement may also help (Kamura 1966, Hillis-Colinvaux 1980) as we have seen in *H. discoidea* and *H. cuneata*, but they are quite rare and undescribed for some taxa (Hillis-Colinvaux 1980).

The utilization of the shape and size of the utricula in surface view under SEM proved to be rewarding allowing species discrimination in some cases if associated with other characters (figures 17-23).

A good example of morphological variability is given by *Halimeda opuntia*, which is well known for its large variation in branching pattern and segment morphology. This species can form extensive mats with entangled branches as well as loose tufts with distichous branching, in which case it can be easily confused with *H. gracilis*. Its segments may be flat, subcylindrical, contorted, auriculate, trilobate and crenulate depending on where it is growing. Because of this variability Barton (1901) proposed to divide this taxon into four forms: *f. cordata*, *f. elongata*, *f. opuntia* and *f. triloba*. Taylor (1960) accepted *f. cordata* and *f. triloba* and added *f. minor* Vickers; however, this division is not supported by our observations for we observed a morphological continuous in large samples and even some specimens with mixed characters.

*Halimeda cuneata*, *H. discoidea*, *H. gracilis*, and *H. tuna*, all from section *Halimeda* Hillis-Colinvaux (1980), are very similar and put together under the *H. tuna* complex (Hillis 1959). *Halimeda cuneata* differs from the others by the presence of a distinctive interconnection node between segments formed by torulous, thick-walled filaments (Hillis 1959, Hillis-Colinvaux 1980). It is possible that some of the
difficulties to species identification may be related to hybridization. However, this is based only on morphology and have no experimental support. This hypothesis has already been suggested by Behar (1972) commenting on the difficulties in distinguishing some specimens of *Halimeda cuneata* from *H. discoidea*. Within this closely related group, *H. discoidea* can be distinguished microscopically by the presence of inflated or subglobose secondary utricles. However, in *H. discoidea* the typical form is attributed to intertidal specimens, whereas the deep-water specimens have been described as *H. discoidea* var. *platyloba* (Taylor 1960). The same applies for *H. tuna*, where the deep-water specimens have been described as *H. tuna* var. *platydisca* or f. *platydysca* (Taylor 1960).

Identification of specimens from deep water is much more complicated because of morphological plasticity (cf. Stark et al. 1969). For example, deep water plants of *H. gracilis* exhibited larger, reniform to subcuneate segments, easily mistaken for the deep-water form of *H. tuna*. This deep-water form of *H. gracilis* was described as *H. gracilis* f. *lata* by Taylor (1950).

The recent introduction of gene sequencing as a tool in the classification of *Halimeda* brings a new set of information (Hillis et al. 1998, Kooistra et al. 2002). This in great part supports the classification based on morphological characters, but not always. For instance in the sectional division of Hillis-Colinvaux (1980) *H. gracilis* appear within the section *Halimeda* (lineage 3 of Kooistra et al. 2002) but in this last work it appears in lineage 4. Molecular data of Kooistra et al. (2002) showed that *H. tuna* from the Atlantic and from the Mediterranean were not nearest neighbor. That paper also casts doubts on the supposed pantropical distribution of several species and suggests allopatric speciation through vicariance in the tropical Atlantic and Indo-Pacific.

On what concerns the distribution of *Halimeda* in Brazil the seven reported species are concentrated on the northeast coast (figure 42). It is worth noting that all species are present on the coast of Bahia state (figure 42). This fits well with the distribution of reefs in Brazil, the largest concentration being in the state of Bahia as well. The southern limit of the genus is in the Cabo Frio region, Rio de Janeiro State (Zeller 1876, Taylor 1930, 1931, Oliveira Filho 1977), just a little north of a well known area of upwelling.

However, it is on the coast of Pernambuco that the largest biomass of *Halimeda* spp. seems to be concentrated, especially due to the thick mats of *H. opuntia*. In this region, dead segments of *Halimeda* form extensive deposits that have been sporadically utilized commercially (Kempf 1970, 1980, Pereira 1974, Oliveira Filho 1981).

As to vertical distribution the genus is well known for having species that go deep in the sublittoral (e.g. Littler & Littler 2000, Stark et al. 1969). In Brazil, *Halimeda gracilis* and *H. discoidea* were found at depths of over 160 m, whereas *H. tuna*, *H. incrassata*, *H. simulans* and *H. opuntia* were found up to 60 m. We have a sample of *H. discoidea* dredged from 260 m; however, there is no guaranty that this species was actually growing at that depth.

In conclusion although we have cleared up some confusions on the identification of the *Halimeda* species in Brazilian herbaria and extended the distribution of several taxa along the coast, we realised that due to morphological convergence in response to habitat and herbivore pressure, the next step would be to check our morphological hypothesis with molecular biology techniques.

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