Diversity of Sulfated Polysaccharides From Cell Walls of Coenocytic Green Algae and Their Structural Relationships in View of Green Algal Evolution

Marina Ciancia1*, Paula Virginia Fernández1 and Frederik Leliaert2,3*

1 Universidad de Buenos Aires, Facultad de Agronomía, Departamento de Biología Aplicada y Alimentos, Cátedra de Química de Biomoléculas, CIHDECAR-CONICET, UBA, Buenos Aires, Argentina, 2 Meise Botanic Garden, Meise, Belgium, 3 Ghent University, Faculty of Sciences, Biology Department, Ghent, Belgium

Seaweeds biosynthesize sulfated polysaccharides as key components of their cell walls. These polysaccharides are potentially interesting as biologically active compounds. Green macroalgae of the class Ulvophyceae comprise sulfated polysaccharides with great structural differences regarding the monosaccharide constituents, linearity of their backbones, and presence of other acidic substituents in their structure, including uronic acid residues and pyruvic acid. These structures have been thoroughly studied in the Ulvales and Ulotrichales, but only more recently have they been investigated with some detail in ulvophytes with giant multinucleate (coenocytic) cells, including the siphonous Bryopsidales and Dasycladales, and the siphonocladous Cladophorales. An early classification of these structurally heterogeneous polysaccharides was based on the presence of uronic acid residues in these molecules. In agreement with this classification based on chemical structures, sulfated polysaccharides of the orders Bryopsidales and Cladophorales fall in the same group, in which this acidic component is absent, or only present in very low quantities. The cell walls of Dasycladales have been less studied, and it remains unclear if they comprise sulfated polysaccharides of both types. Although in the Bryopsidales and Cladophorales the most important sulfated polysaccharides are arabinans and galactans (or arabinogalactans), their major structures are very different. The Bryopsidales produce sulfated pyruvylated 3-linked \( \beta-D \)-galactans, in most cases, with ramifications on C6. For some species, linear sulfated pyranosidic \( \beta-L \)-arabinans have been described. In the Cladophorales, also sulfated pyranosic \( \beta-L \)-arabinans have been found, but 4-linked and highly substituted with side chains. These differences are consistent with recent molecular phylogenetic analyses, which indicate that the Bryopsidales and Cladophorales are distantly related. In addition, some of the Bryopsidales also biosynthesize other sulfated polysaccharides, i.e., sulfated mannans and sulfated rhamnans. The presence of sulfate groups as a distinctive characteristic of these biopolymers has been related to their adaptation to the marine environment.
However, it has been shown that some freshwater algae from the Cladophorales also produce sulfated polysaccharides. In this review, structures of sulfated polysaccharides from bryopsidalean, dasycladalean, and cladophoralean green algae studied until now are described and analyzed based on current phylogenetic understanding, with the aim of unveiling the important knowledge gaps that still exist.

Keywords: coenocytic green algae, sulfated galactan, sulfated arabinan, phylogeny, Ulvophyceae, cell wall

INTRODUCTION

Sulfated polysaccharides encompass a diverse group of anionic polymers, occurring in many different groups of organisms, from macroalgae to mammals, but they are not found in land plants. For marine organisms, as seaweeds, marine invertebrates and sea grasses, sulfated polysaccharides are supposed to be a physiological adaptation to the high ionic strength of the marine environment (Kloareg and Quatrano, 1988; Aquino et al., 2005; Pomin and Mourao, 2008). In addition, they are believed to have important support and protective functions, for example through moisture retention capacity that enhances desiccation resistance (Kloareg and Quatrano, 1988; Arata et al., 2017a). They may also be involved in cell interaction and adhesion, and form a protective barrier against pathogens (Vavilala and D’Souza, 2015; Udayan et al., 2017). Seaweeds, including red (Rhodophyta), green (Chlorophyta), and brown (Phaeophyceae) marine macroalgae, biosynthesize sulfated polysaccharides as a key component of their cell walls (Cosenza et al., 2017). The amounts and structure of these cell wall components vary greatly. Well-known sulfated polysaccharides in seaweeds include galactans (agarans and carrageenans) from red algae, ulvans from green algae, and fucans and fucoidans from brown algae (Usov, 2011; Pangestuti and Kurnianto, 2017; Deniaud-Bouët et al., 2017). Algal sulfated polysaccharides are often complex and they are biosynthesized as heterogeneous mixtures (Pomin and Mourao, 2008), having composition and structure modulated by phylogenetic and environmental factors. From these mixtures, usually extracted from the alga by aqueous solvents, polysaccharide fractions, homogeneous with respect their structure and molecular weight, are difficult to obtain.

The Ulvophyceae include a wide range of marine macroalgae (green seaweeds), but several members also occur in freshwater or moist subaerial habitats. Species in the class display a wide variety of forms, ranging from microscopic unicellular algae to macroscopic multicellular algae, and giant-celled organisms with unique cellular and physiological characteristics (Mine et al., 2008). The origin and early diversification of Ulvophyceae likely took place in the late Tonian and Cryogenian periods, followed by a diversification of the main macroscopic clades in the Paleozoic (Del Cortona et al., 2020). Giant-celled green algae can be categorized into two cytological types, the siphonous and siphonolamalous types (both are also referred to as coenocytic). The siphonous type is characterized by macroscopic algae consisting of a single giant tubular cell generally containing thousands to millions of nuclei. It is present in the orders Bryopsidales and Dasycladales. Their cytoplasm exhibits vigorous streaming, enabling transportation of RNA transcripts across the plant. The siphonolamalous type, that characterizes the Cladophorales, has multicellular bodies composed of multinucleate cells with nuclei organized in regularly spaced cytoplasmic domains (Figure 1).

A solid phylogeny of the Ulvophyceae is important to understand the evolution traits, such as thallus morphology and cell wall structure and components. The relationships among the main clades of ulvophytes have long been uncertain, and even monophyly of the class has been under debate (reviewed by Del Cortona and Leliaert, 2018). A recent genome-scale phylogeny has largely resolved the relationships among the main clades of Ulvophyceae and indicated that macroscopic growth, as well as siphonous organization originated several times independently from unicellular ancestors (Del Cortona et al., 2020). Figure 2 shows our current understanding of phylogenetic relationships between the different orders within the Ulvophyceae.

Species in the Ulvophyceae biosynthesize sulfated polysaccharides with important structural differences regarding the monosaccharide constituents, linearity of their backbones, presence of methoxyl groups, uronic acid residues, as well as acidic substituents of neutral monosaccharide units, including pyruvic acid, which contribute with sulfate groups to the negative charge of these polymers (Percival, 1979; Lahaye and Robic, 2007; Fernández et al., 2014; Stiger-Pouvreau et al., 2016; Kidgell et al., 2019).

An early classification of this structurally heterogeneous polysaccharide group, that is still very useful and generally used today, was based on the presence of uronic acid residues in their structures (Percival, 1979; Fernández et al., 2014; Stiger-Pouvreau et al., 2016).

Sulfated polysaccharides from the Ulvales and Ulotrichales have been thoroughly studied; they belong to the group comprising significant quantities of uronic acids. There are some major differences between the structures of those from both orders. Ulvans, the sulfated polysaccharides from the Ulvales, comprise major amounts of rhamnose and uronic acids disaccharidic repeating units. Many different oligosaccharides were obtained by hydrolysis of ulvans, a major structure found was 4-O-β-D-glucuronosyl-L-rhamnose. Also important quantities of other sugars were detected, as xylose, glucose, and iduronic acid (Lahaye and Robic, 2007). Sulfated polysaccharides from the Ulotrichales, as those from some species of the genus *Monostrama* and *Gayralia oxyperma* are also constitued by major amounts of rhamnose and glucuronic acid, but they have a
Cladophorales fall in the same group, in which this acidic structures, sulfated polysaccharides of the orders Bryopsidales and are beginning to be understood. In agreement with their chemical investigated. The study of Percival & Smestad (1972) suggests that However, cell wall components of Dasycladales remain poorly biosynthesize polysaccharides belonging to the same group. In particular, mannans were found to be the major fibrillar polysaccharides in Codium species, while major quantities of 3-linked β-δ-xylans, plus small amounts of cellulose were found in Bryopsis and Dichotomosiphon (Macda et al., 1966; Percival and McDowell, 1981; Fernández et al., 2014). It has been shown that the major fibrillar polysaccharides can vary in the different life stages of a single species. For example, sporophyte macrothalli of the genus Derbesia have fibrillar mannans as major components, while gametophyte microthalli biosynthesize xylans. Gametophyte macrothalli from the genus Bryopsis biosynthesize important quantities of xylans, while sporophyte microthalli produce mannans (Huizing and Rietema, 1975; Wutz and Zetsche, 1976; Huizing et al., 1979).

The Cladophorales are found in diverse habitats, ranging from marine habitats (occurring in the intertidal down to depths of more than 100 meters) to a wide range of freshwater habitats (Leliaert et al., 2012; Škaloud et al., 2018). Their cell walls are composed of cellulose I as major fibrillar polymer, with parallel microfibrils in numerous lamellae, of high crystallinity, lacking amorphous regions characteristic of plant cellulose (Zulkifli et al., 2013). In the cell walls of Pithophora roettleri, chitin was present in small amounts (>10%), in addition to cellulose (Pearlmutter and Lembi, 1980).

![Figure 1](image-url)
SULFATED POLYSACCHARIDES FROM COENOXYTIC GREEN ALGAE

Bryopsidales

The Bryopsidales consist of three main clades, corresponding to the suborders Bryopsidineae, Halimedineae, and Ostreobineae (Figure 3), which are characterized based on differences in thallus morphology, reproduction and plastid types (Hillis-Colinvaux, 1984; Cremen et al., 2019).

The Bryopsidineae (including Bryopsis, Codium, and Derbisia) comprise the families Bryopsidaceae, Codiaceae, Derbesiaceae, and Pseudobryopsidaceae, with species inhabiting temperate to tropical marine waters. The Halimedineae (including Caulerpa, Pencillia, Halimeda, and Udotea), comprise the families Caulerpaceae, Halimedaceae, and Dichotomosiphonaceae, and are generally restricted to tropical and subtropical habitats. However, exceptions to these general distribution patterns occur, including the presence of some invasive Caulerpa species in temperate waters. Several species in this subclass (mainly in the Halimedaceae) are heavily calcified.

The structure of sulfated polysaccharides from the suborders Bryopsidineae and Halimedineae will be reviewed. No data on sulfated polysaccharides is currently available for the Ostreobineae.

Bryopsidineae

The most widely studied genus with respect to its sulfated polysaccharides is Codium.

Love and Percival (1964) studied the water extracts from Codium fragile, which included 3-linked β-L-arabinopyranose and β-D-galactopyranose units. Sulfate was found on C-4 or C-6 of the galactose units, and on C-2 or C-3 of the arabinose residues. It was suggested that these sugars could be present in the same molecule, being arabinogalactans. More recently, extracts from Codium species were isolated and, eventually, fractionated and partially characterized because of their interest as anticoagulants (Ciancia et al., 2010). Most of these fractions contained galactose as major monosaccharide constituent, but a few contained arabinose as the major sugar, or had both.

A key work for understanding the actual structure of galactans from Codium was published by Bilan et al. (2007). In this paper, the structure of a sulfated, pyruvylated (1→3)(1→6)-β-D-galactan obtained from C. yezoense was exquisitely studied by chemical and spectroscopic methods. Acyclic galactans of this kind were found in many other Codium species, including C. fragile (Ciancia et al., 2007; Estevez et al., 2009; Ohta et al., 2009; Li et al., 2016), C. vermiculara (Ciancia et al., 2007; Fernández et al., 2013), C. isthmocladum (Farias et al., 2008; Araujo Sabry et al., 2019), C. decorticatum (Fernández et al., 2015), and C. divaricatum (Li et al., 2015). They showed highly ramified structures, which contained linear backbone segments of 3-linked β-D-galactopyranose residues connected by (1→6)-linkages, some of 3-linked residues were substituted at C-6, by short oligosaccharide chains also containing (1→3)- and (1→3,6)-linkages or by single β-D-galactopyranose residues. Sulfate groups were found mainly at C-4 and in minor amounts at C-6, confirming the study of Love and Percival (1964). The most unusual feature of these water-soluble galactans was the high pyruvate content, which was found to form mainly five-membered cyclic ketals with O-3 and O-4 of the non-reducing terminal galactose residues (S-isomer). However, a minor part of pyruvate formed six-membered cyclic ketals with O-4 and O-6 (R-configuration) (Bilan et al., 2006). These galactans are the only known algal polysaccharides containing five-membered cyclic pyruvate ketals studied until now. A possible structure for a fragment containing all the main structural features is shown in Figure 4A.

Although similar, galactans from different Codium species have important interspecific differences regarding the proportion of their structural units. For example, in samples of C. fragile...
from the Patagonian coast, 4,6-disulfated β-D-galactose units were found in significant quantities, and the presence of terminal 3,4-pyruvylated β-D-galactose 6-sulfate units was suggested (Estevez et al., 2009). The occurrence of the latter unit was proved by negative-ion electrospray tandem mass spectrometry of oligosaccharides obtained from the galactan from C. divaricatum in mild acid conditions (Li et al., 2016), and it was also detected recently in C. isthmocladum (Araujo Sabry et al., 2019). In the galactan from C. decorticatum, almost half of the pyruvate was forming a 6-membered ring with O-4 and O-6 of 3-linked β-D-galactose units (Fernández et al., 2015).

Sulfated arabinans with high anticoagulant activity were found in some Codium species, including C. latum, C. dwarkense, and others (Uehara et al., 1992; Siddhanta et al., 1999; Hayakawa et al., 2000), suggesting that these polymers, generally highly sulfated, are usual components of Codium cell walls. Later, Ciancia et al. (2007) showed that the major structural units in these polymers were 3-linked β-L-arabinopyranose highly sulfated on C-4, or C-2 and C-4 (Figure 4D). In particular, an almost persulfated linear arabinan was isolated from C. vermilara by precipitation in 0.1 M KCl (molar ratio arabinosesulfate 1:1.8). This arabinan had a high anticoagulant activity by complex mechanisms, comprising direct and indirect inhibition of thrombin (Fernández et al., 2013). Arabinans from C. decorticatum were less sulfated and showed a high degree of branching at C-2, at least in part with single stubs of arabinose (Fernández et al., 2015). In the case of C. fragile, not only pyranosic 3-linked sulfated β-L-arabinans, but also furanosic non sulfated α-L-arabinan structures were detected, and they were associated to the presence of hydroxyproline rich glycoproteins (HRGP) (Estevez et al., 2009).

A third sulfated polysaccharide type was also found in water soluble extracts from Codiunm species and corresponded to 4-linked β-D-mannans, similar to those found as fibrillar components in these seaweeds, as well as plant mannans and galactomannans, but sulfated on C-2 and, in a lesser extent, on C-6 (Ciancia et al., 2007; Fernández et al., 2012; Fernández et al., 2015) (Figure 4E). A paper reporting immunomodulatory activity of a mannan from C. fragile postulated a structure corresponding to a 3-linked mannan comprising α- and β-units, sulfated on C-4 of the latter units (Tabarsa et al., 2013). However, the results, including NMR (nuclear magnetic resonance) spectroscopy and methylation analyses, suggest a structure similar to that found for the other Codiunm species.

As far as we know, the only other genus in this suborder for which sulfated polysaccharides have been studied in detail is Bryopsis (Ciancia et al., 2012). From a room temperature water extract, a linear 3-linked b-D-galactan, partially sulfated, mainly on C-6 and partially pyruvylated was isolated from B. plumosa. In this species, the pyruvylated units were part of the linear
backbone, giving 4,6-O-(1´-(R)-carboxy)ethylidene-β-D-galactopyranose units (Figure 4B). The other polysaccharide obtained with water at room temperature was a furanosic, non-sulfated, mostly 2-linked β-L-arabinan, with ramifications on C-5. This structure is different to those detected in C. fragile, and further work should be done to confirm it. In addition, a rhamnan structure (17.4 mol% rhamnose) was detected as one of the major constituents in the hot water extract from the same seaweed. It comprised mainly 3-linked, possibly partially 4-sulfated, 1-rhamnose units, but also 2-linked 1-rhamnose residues. The fact that small amounts of uronic acids were present in these extracts, suggested that these rhamnans could be structurally related to sulfated polysaccharides from green seaweeds of the order Ulotrichales (Cassolato et al., 2008; Liu et al., 2018).

**Halimedineae**

Several species in this suborder are calcified and give extremely low yields in sulfated polysaccharides, which may be the reason for the lack of information about their sulfated polysaccharides. As far as we know, the only structural study about sulfated polysaccharides in this algal group is that of Arata et al. (2015), who studied the water soluble extracts from three species, *Penicillus capitatus*, *Udotea flabellum* and *Halimeda opuntia*.
(Halimedaceae). They found that sulfated and pyruvylated β-D-galactans were the only soluble sulfated polysaccharides obtained in significant yields. Their backbones comprise 3-, 6-, and 3,6-linkages, constituted by major amounts of 3-linked 4,6-O-(1´-carboxy)ethylidene-D-galactopyranose units in part sulfated on C-2. Also possibly terminal 4,6-O-(1´-carboxy)ethylidene-D-galactopyranose residues were present in their structures, but in much lower amounts. Sulfation on C-2 was not found in galactans from other seaweeds of the Bryopsidales. In addition, a complex sulfation pattern, comprising 4-, 6-, and 4,6-disulfated galactose units was found, as in galactans from other species of this order (Figure 4C).

Mackie and Percival (1961) reported some structural features about the polysaccharides from three species of Caulerpa, C. filiformis, C. racemosa, and C. sertularioides. They all contained galactose as major monosaccharide, and significant quantities of mannose and xylose. Different batches of C. filiformis were investigated and in one of them, arabinose was also present. The authors attributed this difference to a seasonal variation, or to the fact that the latter sample was obtained in milder extraction conditions. They suggested that both galactose and mannose units were 3-linked, based on their resistance to periodate oxidation.

The sulfated heteropolysaccharide isolated from Caulerpa taxifolia comprised galactose, mannose, and xylose in molar ratio 16.4:5:1. 4-Linked xylose, 6-linked galactose, 4-linked mannose units, and non-reducing galactose end groups, which were all devoid of sulfate, were found. In addition, 4-linked galactose units sulfated at C-3 were also present. An oligosaccharide was isolated from the acid hydrolyzate, assigned to 4-O-(D-mannopyranosyl-4-O-D-galactopyranosyl)-D-mannopyranose (Prasada Rao and Venkata Rao, 1986). Later, a water soluble acidic heteroglycan sulfate, comprising arabinose, xylose, and galactose, was obtained from Caulerpa racemosa with a molecular mass of 80 kDa. It contained, inter alia, 3-linked galactose, terminal- and 4-linked xylose, and 4- and 3,4-linked arabinose residues. Sulfate groups, when present, were located at C-3 of 4-linked arabinose and C-6 of 3-linked galactose units (Chattopadhyay et al., 2007a).

Recently, a xylogalactomannan fraction containing galactose, mannose and xylose in molar ratio 2.4:2.2:1, and 21% sulfate was isolated from C. lentillifera. It consisted in 3-linked and terminal β-D-galactopyranose units, the former partially sulfated on C-6, 4-, 2,4-, and 2-linked β-D-mannopyranose units, the latter sulfated on C-3, and 4-linked 3-sulfated, and terminal β-D-xylpyranose units (Sun et al., 2018; Sun et al., 2020). This sample was reported to have a molecular weight of 3877.8 kDa, which is quite high for a sulfated polysaccharide, leading us to speculate that it could comprise several aggregated polysaccharides.

A water-soluble polysaccharide was obtained by hot-water extraction from Caulerpa racemosa var. peliata by Hao et al. (2019). After purification, it contained mannose as major monosaccharide component (92% of the total carbohydrates). The structural analysis reported a polysaccharide fraction comprising a backbone of 6-linked α-D-mannopyranose residues partially sulfated on C-3, with some 4-linked and 2-linked α-D-mannopyranose residues, and side chains comprising 4-linked β-D-galactopyranose residues.

Several papers appeared in the last few years, which reported biological activity of polysaccharides obtained from Caulerpa species, but these products were only characterized with respect to their molecular weight and monosaccharide composition, and no, or only little structural information was given (Chaves Filho et al., 2019; Albuquerque Ribeiro et al., 2020; Long et al., 2020; Zhang et al., 2020).

The lack of information about sulfated polysaccharides from such an important and widely distributed algal genus, comprising at least 75 species, some of which of them having invasive nature (Galil, 2007; Pierucci et al., 2019), is noteworthy. Also noteworthy is the variety of structures proposed in the few investigations published for the different species of the same genus. A revision of the structural features of cell wall polysaccharides from a variety of different macroalgae shows that a certain genus usually produces polysaccharides with only some variations from a general common structural pattern, which is characteristic for it. A lot of work is needed to clarify the structures of the whole system of sulfated polysaccharides biosynthesized by these organisms, as the small number of structural studies reported were usually carried out on a particular polysaccharide fraction, and there is not enough information about the other sulfated polysaccharides biosynthesized by the studied species. Likely, the whole polysaccharide system biosynthesized by Caulerpa species comprises variable quantities of sulfated β-D-galactans, pyranosyl arabinans, and mannans. Xylose could be part of the galactan or arabinan structures, or another polymer.

**Dasycladales**

Species in the Dasycladales have a siphonous architecture, with symmetrical thalli encrusted with calcium carbonate. Siphons contain either one macronucleus or millions of small nuclei. Species occur in marine tropical seas. Two families have traditionally been recognized based on differences in the reproductive structures, the Polyphysaceae (including Acetabularia and some other genera), and the paraphyletic Dasycladaceae (including genera like Bornetella, Cypodium, and Noomeris) (Verbruggen et al., 2009).

Only a few reports are available about sulfated polysaccharides from the Dasycladales. Percival and Simestad (1972) studied the structural features of water soluble polysaccharides from stalks and caps of Acetabularia crenulata. They were able to determine the presence of 3-linked galactose 4-sulfate in major amounts, and also 6-sulfate, and 2-linked 1-rhamnose, as the main structural features. Glucuronic acid, galactose, and rhamnose were present as end groups, indicating a highly branched molecule. Glucuronic acid was linked to both rhamnose and galactose. In addition, they found that galactose residues were linked together, and some galactoses were 4-O-methylated.

Cell walls from rhizoids, stalks, hairs, hair scars, apical septa, gametophores and gametangia from A. acetabulum were prepared, but the polysaccharides were not separated according to their water solubility, but analyzed directly by NMR spectroscopy and monosaccharide composition (Dunn et al., 2007). In these
conditions, the major monosaccharide component was in all cases mannose, arising in major amounts from the fibrillar cell wall polysaccharide components. Glucose, deriving from cellulose, was also detected, mainly in gametophores and gametangia (haploid structures). Additionally, galactose, rhamnose, xylose, and arabinose were found in different but always small amounts. Although the authors performed methylation analysis, the predominance of mannose in these extracts precludes further conclusions.

The very limited information about these polymers makes it impossible to draw conclusions, however, we speculate that these seaweeds could biosynthesize both sulfated 3-linked β-D-galactans or arabinogalactans and glucuronorhamnans. This speculation is based on the results of methylation analysis of samples from different parts of the thallus from Acetabularia acetabulum, that show, in addition to important amounts of mannans and cellulose, other monosaccharides, as rhamnose and glucuronic acid (Dunn et al., 2007) and the detail paper by Percival and Smeastad (1972), which suggests the presence of 3-linked 4-sulfated galactan structures, similar to those of Codium, in Acetabularia crenulata. These polysaccharides could be distributed in the cell walls of each seaweed structure in different quantities, according to their requirements. Moreover, it was found that the amount of rhamnose in various wall regions roughly correlated with the degree of flexibility of that anatomical region. The proportion of rhamnose was the greatest in hairs, the most flexible region, often undulating in the moving seawater (Dunn et al., 2007). These authors associated the monosaccharide composition determined with cell wall polymers from flowering plants, but they can also be associated to cell wall polysaccharide components from phylogenetically closer organisms, as other green algae.

**Cladophorales**

Species of Cladophorales occur in a wide variety of marine, brackish and freshwater habitats from tropical to polar environments. Thallus organization ranges from simple, branched or unbranched filaments to more complex architectural types. The order comprises five main family-level clades (Figure 5). The two largest clades are the Cladophoraceae, including genera with relative simple thallus morphology such as *Cladophora, Chaetomorpha* and *Rhizoclonium*, and occurring in marine to freshwater habitats, and the Siphonocladiaceae clade, including a wider range of thallus architectures, and occurring in marine habitats. Cell wall polysaccharides have, as far as we know, only been investigated in species of the Cladophoraceae.

The extensive studies of Percival and coworkers about cell wall polysaccharides from green seaweeds include the determination of fine structural details of sulfated polysaccharides from *Cladophora rupestris*, which comprised arabinose, galactose, xylose, rhamnose, and glucose in molar ratio of 3.7:2.8:1.0:0.4:0.2, as well as 8% protein and 19.6% sulfate (as SO₃Na) (Fischer and Percival, 1957). A highly branched structure, with xylose and galactose units at the non-reducing ends of the side chains and galactose, arabinose, and rhamnose residues occurring in the inner part of the molecules was proposed. The evidence of 6-linked and/or 6-sulfated galactofuranose units obtained in this study is noteworthy, as furanosic galactose containing polysaccharides were only found in some green microalgae species associated with lichens, as *Trebouxia* sp. and *Myrmenia biotarellae* (Trebouxiales, Trebouxiophyceae). These polysaccharides have a main backbone of β-D-galactofuranose units, with different side chains, constituted by single stubs of β-D-galactofuranose and/or more complex branched structures, which comprise, in some cases, α-L-rhamnopyranose units (Cordeiro et al., 2005; Cordeiro et al., 2012; Cordeiro et al., 2013).

Partial hydrolysis experiments of the sulfated polysaccharides from *C. rupestris* led to the separation and characterization of the following fragments: L-arabinose 3-sulfate, D-galactose 6-sulfate, 3-linked and 6-linked D-galactobioses, 4- or 5-linked L-arabinobiose 3-sulfate, 4-linked D-xylolbiose, a mixture of trisaccharides containing sulfated galactose and arabinose, and a mixture of pentasaccharides in which the molar ratio of arabinose to galactose was 4:1 (Hirst et al., 1965; Johnson and Perival, 1969; Bourne et al., 1970). In addition, preliminary studies were also done on the sulfated polysaccharides from Chaetomorpha capillaris and *Ch. linum*, which gave a predominance of arabinose as monosaccharide component (Hirst et al., 1965). For these seaweeds, no further studies were carried out at that time due to the difficulties to get enough material. Later, it was found that arabinose was also the predominant monosaccharide component of the sulfated polysaccharides from Ch. anteninna (58% of the purified polysaccharide), and it was in the pyranose form, giving 4-linked arabinopyranose partially sulfated on C-2, while 3- and 4-linked galactopyranose units were also present in important amounts (39%) and small quantities of 4-linked rhamnose were also detected (Venkata Rao and Sri Ramana, 1991).

The same authors studied the sulfated polysaccharides from *Cladophora socialis* obtained by extraction with dilute acid (Sri Ramana and Venkata Rao, 1991). A molar ratio galactose: arabinose:xylose of 4.5:3.0:1.0 and 16.9% of sulfate were determined. In this case, the 4-linked arabinose units were found to be 3-sulfated, but no galactose in the furanose form was detected, we think it could have been lost due to its lability in acidic extraction conditions. In addition, galactose units were found to be 3-linked and sulfated on C-4 or C-4 and C-6.

The studies carried out by Arata et al. (2016) on the water soluble sulfated polysaccharides from the seaweed *Cladophora falklandica* were also in agreement with the classical work (Percival and McDowell, 1981), but the authors were able to determine some structural features not reported before using modern spectroscopic methods in addition to the chemical determinations. It was found that the major sulfated polysaccharides from this species were xylogalactoarabinans constituted by a backbone of 4-linked β-L-arabinopyranose units partially sulfated mainly on C-3 and also on C-2. Besides, partial glycosylation mostly on C-2 with single stubs of β-D-xylolpyranose, or single stubs of β-D-galactofuranose or short chains comprising (1→5)- and/or (1→6)-linkages, was also found (Figure 6). In addition 3-linked β-D-galactopyranose units sulfated on C-6 were also detected in minor amounts in...
some of the fractions. It was not possible to establish whether they were part of the major polysaccharides or if they constituted another polysaccharides present in minor amounts. Small amounts of rhamnose were also present in all these fractions.

Similar polysaccharide structures were found for the freshwater *Cladophora surera*, although only traces of galactose in the furanosic form were detected. The presence of sulfated polysaccharides in a freshwater green macroalga of the Cladophorales could be, in this case, an adaptation to transient desiccation and changes in ionic strength of its environment.

While writing this review, a paper about the structure of a sulfated polysaccharide fraction from *Chaetomorpha linum* was published, with a new structure constituted by 6-linked β-D-galactopyranosyl and 5-linked α-L-arabinofuranosyl residues with sulfate groups on C-2 or C-3 of the latter units and on C-2 or C-4 of 6-linked β-D-galactopyranosyl residues (Qin et al., 2020). This structure was determined by chemical and spectroscopic analyses. As only one fraction was studied, it is not known if the remaining carbohydrate material has similar structures to those published before for other phylogenetically close algal species.

A pyranosic 4-linked β-L-arabinan sulfated on C-3 was also isolated from the green seaweed *Ulva (Enteromorpha) clathrata* (Ulvala) in a pure form, and only minor amounts of rhamnose and glucuronic acid were found (Qi et al., 2012). This arabinan has the same backbone and major sulfation pattern as that of *Cladophora* species, as well as that reported for *Caulerpa racemosa* (Chattopadhyay et al., 2007a, see above). This result is unexpected, considering that it is well documented that green algae from the Ulvales biosynthesize uronate-rich sulfated polysaccharides, known as ulvans, composed of rhamnose, xylose, glucuronic acid, and iduronic acid (Lahaye and Robic, 2007; Cosenza et al., 2017), but not arabinose. However, there is no doubt regarding the isolated structure, determined by the chemical and spectroscopic analyses presented in this paper. Moreover, as indicated by the authors, this high arabinose-containing sulfated polysaccharide has different chemical characteristics from the sulfated polysaccharides isolated from species of the Ulvales, which should belong to the uronic acid rich polysaccharide group, as defined by Percival (1979). In the last years, many reports confirmed that seaweeds of this order, including *Ulva lactuca*, *U. intestinalis*, *U. prolifera*, *U. linza*, and *U. compressa*, biosynthesize major amounts of sulfated glucuronorhamnans (Chattopadhyay et al., 2007b; Lahaye and Robic, 2007; Wang et al., 2013; Yu et al., 2017; Li et al., 2018). In these reports, arabinose was not detected, or if so, only in trace amounts. If the algal species determination was correct, a revision of the classification of sulfated polysaccharides from green seaweeds regarding their phylogenetic positions could be evaluated. In addition, it should be considered that only the major fraction obtained from the hot water extract of *U. clathrata* by anion exchange chromatography was analyzed, and no information was given regarding the other polysaccharides of the extract (Qi et al., 2012).

Another possible exception was reported for the structure of sulfated polysaccharides from *Spongomorpha indica* (Ulotrichales). Their backbone was composed by 4-linked galactose units, terminal, 3-linked and 2-linked arabinofuranose units, as well as 4-linked arabinopyranose units were detected, and xylose was present as branches. Sulfate groups were found on some of the arabinose units at C-2 and on some of the galactose units at C-2 and C-3 (Venkata Rao et al., 1991).

**CONCLUDING REMARKS**

By reviewing the diversity of sulfated polysaccharides from cell walls of coenocytic ulvophytes in the orders Bryopsidales, Dasycladales, and Cladophorales in view of their phylogenetic relationships, we identified important knowledge gaps related to the diversity of sulfated polysaccharides in green seaweeds. The knowledge on the structures of cell wall polysaccharides, especially the sulfated polysaccharides, biosynthesized by them is very limited, even for many common and widely distributed species, and, in very few cases, the whole system of cell wall...
sulfated polysaccharides was investigated. In addition, the species that have been investigated are distributed unevenly among the orders, families and genera of the Ulvophyceae. Several families within the orders Bryopsidales, Dasycladales, and Cladophorales have not been studied, and the same is true for several smaller orders of Ulvophyceae, including the Trentepohliales, Ignatiales, Scotinosphaerales, Chlorocystidales, and Oltmannsiellopsidales (Figure 2). This is because most of the studies were not carried out with a phylogenetic perspective, but with the aims of finding biologically active compounds that could have interest as potential pharmacological drugs, or with other commercial interests. Information on these understudied groups, however, will be invaluable to reconstruct the evolution of sulfated polysaccharides in the Ulvophyceae, and could potentially result in the detection of novel bioactive compounds. This is plausible given the antiquity of the ulvophycean lineages, which likely diverged in the Proterozoic (Del Cortona et al., 2020), providing plenty of time to evolve novel bioactive compounds. In addition ulvophycean species occur in a wide diversity of habitats, ranging from fully marine environments to freshwater and strictly terrestrial habitats (Škaloud et al., 2018), which likely has an important effect on polysaccharide structure.

Until now, no useful rheological properties were found for these sulfated polysaccharides, as those of galactans from red seaweeds (carrageenans and agarose) that make these compounds industrially produced as hydrocolloids (Gomez et al., 2020). Moreover, their small yields make this possible application unlikely. Conversely, many studies have sought to find different biological activities of these compounds. For example, anticoagulant activity of polysaccharides from Codium species was thoroughly investigated for many years (Ciancia et al., 2016; Araujo Sabry et al., 2019). More recently, anticoagulant activity of sulfated polysaccharides from other coenocytic green algae was also explored, including detailed studies about their mechanisms of action (Arata et al., 2015; Arata et al., 2016; Arata et al., 2017b; Qin et al., 2020). In addition, some other interesting biological activities were found, including proinflammatory activity (Tabarsa et al., 2013), anti-inflammatory activity (Sun et al., 2020), macrophage activation (Surayot et al., 2016), antioxidant activity (Hamzaoui et al., 2020), immunostimulatory activity (Lee et al., 2010; Sun et al., 2018; Hao et al., 2019), and antiviral activity (Pujol et al., 2012). Thus, it seems that the potential application of these polysaccharides could arise from their biological action.

Although a lot of work is still needed, the available information shows important characteristics that define the different coenocytic green algal groups, up to order and also the family level. Table 1 gives a picture of the possible generalizations that can be made at this stage.

Cell walls are very complex and diverse supramolecular structures. Their macromolecular components and arrangement have been elucidated for some groups of organisms as the flowering plants (Gibeaut and Carpita, 1993; Park and Cosgrove, 2012). Conversely, algae comprise of many distantly related groups of organisms, for some of which cell wall structures are better understood than others (Popper et al., 2011; Domozych et al., 2012). Particularly, cell wall architecture from green macroalgae of the Ulvophyceae has been investigated in only a few cases (Lahaye and Robic, 2007; Estevez et al., 2009; Fernández et al., 2010; Ciancia et al., 2012; Fernández et al., 2014; Fernández et al., 2015).

It is evident from Table 1 that some important structural features of sulfated polysaccharides are shared between the Cladophorales and Bryopsidales, in spite of their phylogenetic
distance, as the presence of 3-linked β-D-galactans and 3- or 4-linked β-L-arabinans that are not usually found in those from the Ulvales and Ulotrichales.

Pyranosic 3-linked β-D-galactans are found in many different organisms, as cell wall arabinogalactan proteins (AGP) and pectins from higher plants, but pyranosic β-L-arabinans are not. Moreover, L-arabinose is a constituent of many different plant cell wall components, but, although the pyranose form of L-arabinose is thermodynamically more stable, in these polymers, it occurs mostly in the furanose form (Fernández et al., 2013). More precisely, L-arabinopyranose units were found as part of side chains in Type II arabinogalactans from pectins and in some xyloglucans from flowering plants (Caffal and Mohnen, 2009; Tuomivaara et al., 2015), usually as terminal non reducing units, but not as oligo- or poly-saccharide sequences. So, it is tempting to speculate that pyranosic arabinans are a specific character of cell walls from ancestor organisms of the Ulvophyceae that are still present in some lineages, but were lost during evolution in some others, and it could be related to the evolution of green seaweeds and land plants. There are other structural features that are characteristic of a certain order, as the presence of pyruvic acid substituting some of the β-D-galactose units in β-D-galactans from the Bryopsidales, or the presence of β-D-galactofuranose in side chains of arabinans from the Cladophorales.

In the case of the Bryopsidales, there is enough information to look further into the suborder and family level of structural relationships between sulfated galactans. The presence of the pyruvic acid substituent of β-D-galactans forming a five-membered cyclic ketal with O-3 and O-4 of non-reducing terminal galactose residues was only found in the Codiaeaceae, while pyruvic acid ketals forming a six-membered ring with O-4 and O-6 of some of the 3-linked β-D-galactose units of the galactan backbone, were found in all the species of Bryopsidales in which this structural unit was searched for (Fernández et al., 2014).

In this review, the major sulfated polysaccharides have been considered. In most, if not in all, cases, small amounts of other monosaccharide constituents suggest that there could be some other polysaccharides, minor constituents of the cell wall, the nature of which remains elusive. For example, when the sulfated polysaccharide systems from Cladophora falklandica and C. surera were studied, minor amounts of rhamnose were detected (Arata et al., 2016; Arata et al., 2017a). It is not known if it is derived from a hmannan present in minor amounts, or if it is a minor constituent of the major water soluble polysaccharides obtained from these algae. In addition, a sulfated rhamnan structure was detected in the hot water extract from Bryopsis plumosa in considerable amounts (Ciancia et al., 2012). These observations can only be made when all the sulfated

| Order                  | Cytomorphological type | Major fibrillar component | Uronic acids | Backbone | Sulfated polysaccharides |
|------------------------|------------------------|---------------------------|--------------|----------|-------------------------|
| Bryopsidales           | Siphonous              | (1→4)-β-mannans or (1→3)-β-xylans and small amounts of cellulose | Absent (or present in very low quantities) | Galactan | -3,6-linked-β-galactan sulfated on C-4 and C-6, 3,4 (also 4,6-) pyruvylated |
|                        |                        |                           |              |          | -Pyranosic 3-linked-β-arabinan sulfated on C-2 and C-4, or on C-4, or from HRGPs |
|                        |                        |                           |              | Arabinan | -Pyranosic 4-linked-arabinan sulfated on C-3 |
|                        |                        |                           |              | Other    | -6-linked-β-mannan (galactomannan) |

**TABLE 1** General characteristics of cell wall polysaccharides from coenocytic green algae.
polysaccharides from a certain species are investigated, and could make us speculate that ulvophyte green algae have the enzymes to biosynthesize all of these polysaccharides, but only produce some through biosynthetic regulating factors yet to be unveiled. This speculation could explain the presence of polysaccharides others than those expected in some cases, as in Ulva clathrata and Spongomorpha indica (Venkата Rao et al., 1991; Qi et al., 2012).

Another possibility in view of the latest findings regarding evolution of ulvophyte green algae (Del Cortona et al., 2020) is that not only morphological characters, such as siphonous thallus architecture, but also some structural features of the cell wall, including some sulfated polysaccharides structures, could have appeared independently during evolution of these green algae as a result of similar environmental selective pressures, or more likely as a response to increasingly enlarged cells in the course of evolution of the Bryopsidales and Dasyycladales.

AUTHOR CONTRIBUTIONS
MC and PF analyzed and discussed the polysaccharides structures. FL analyzed and discussed the phylogenetic relationships. MC programmed the review structure. MC and FL integrated the whole analysis and wrote the manuscript.

FUNDING
This work was supported by the National Research Council of Argentina (CONICET) (PIP 11220130100762CO 2014–2016, PU-E 2016 2292016010068CO) and the University of Buenos Aires (UBACYT 2018–2021, 20020170100347BA).

ACKNOWLEDGMENTS
MC and PF are Research Members of the National Research Council of Argentina (CONICET).

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.554585/full#supplementary-material

REFERENCES
Albuquerque Ribeiro, N., Vasconcelos Chaves, H., Conceição Rivanor, R. L., Rochado Val, D., Limade Assis, L., Dantas Silveira, F., et al. (2020). Sulfated polysaccharide from the green marine algae Caulerpa racemosa reduces experimental pain in the rat temporomandibular joint. Int. J. Biol. Macromol. 150, 253–260. doi: 10.1016/j.ijbiomac.2020.01.272
Aquino, R. S., Landeira-Fernandez, A. M., Valente, A. P., Andrade, L. R., and Mourão, P. A. (2005). Occurrence of sulfated galactans in marine angiosperms: evolutionary implications. Glycobiology 15, 11–20. doi: 10.1093/glycob/cvh138
Arata, P. X., Quintana, I., Ilafo, M. P., and Ciancia, M. (2016). Novel sulfated xylolactoarabinins from green seaweed Cladophora falklandica: chemical structure and action on the fibrin network. Carbohydr. Polym. 154, 139–150. doi: 10.1016/j.carbpol.2016.07.088
Arata, P. X., Alberghina, J., Confolonieri, V., Errea, M. I., Estevez, J. M., and Ciancia, M. (2017a). Sulfated polysaccharides in the green macroalga Cladophora sarea not linked to salinity adaptation. Front. Plant Sci. 8, 1927. doi: 10.3389/fpls.2017.01927
Arata, P. X., Genoud, V., Lauricella, A. M., Ciancia, M., and Quintana, i. (2017b). Alterations of fibrin networks mediated by sulfated polysaccharides from green seaweeds. Thromb. Res. 159, 1–4. doi: 10.1016/j.thromres.2017.09.014
Araujo Sabry, D., Lima Cordeiro, S., Ferreira Silva, C. H., Cunha Farias, E. H., Mourão, P. A. (2019). Pharmacological prospection and structural characterization of two purified Sulphated polysaccharides from tropical green seaweeds of the order Bryopsidales. Carbohydr. Polym. 222, 115010. doi: 10.1016/j.carbpol.2019.115010
Bilan, M. I., Vinogradova, E. V., Shashkov, A. S., and Usov, A. I. (2006). Isolation and preliminary characterization of a highly pyruvylated galactan from Codium yezoense (Bryopsidales, Chlorophyta). Bot. Mar. 49, 259–262. doi: 10.1515/BOT.2006.029
Bilan, M.II, Vinogradova, E.V., Shashkov, A. S., and Usov, A.II (2007). Structure of a highly pyruvylated sulfated galactan from the Pacific green alga Codium yezoense (Bryopsidales, Chlorophyta). Carbohydr. Res. 342, 586–596. doi: 10.1016/j.carres.2006.11.008
Boedeker, C., O’Kelly, C. J., Star, W., and Lelisrt, F. (2012). Molecular phylogeny and taxonomy of the Aegagropilopsis clade (Cladophorales, Ulvophyceae), including the description of Aegagropilopsis gen. nov. and Pseudocladosiphora gen. nov. J. Phycol. 48, 808–825. doi: 10.1111/j.1529-8817.2012.01455.x
Bourne, E. J., Johnson, P. G., and Perival, E. (1970). The water-soluble polysaccharides of Cladophora rupestris. Part IV. Autohydrolysis, methylation of the partly desulphated material and correlation with the results of Smith degradation. J. Chem. Soc. C. 1561–1569. doi: 10.1039/37/00015161
Cafall, K. H., and Mohnen, D. (2009). The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr. Res. 344, 1879–1900. doi: 10.1016/j.carbpol.2009.05.021
Casaliotti, J. E. F., Noseda, M. D., Pujol, C. A., Pellizzari, F. M., Damonte, E. B., and Duarte, M. E. R. (2008). Chemical structure and antiviral activity of the sulfated heterorhamnan isolated from the green seaweed Garryalia oxyperma. Carbohydr. Res. 343, 3085–3095. doi: 10.1016/j.carbpol.2008.09.014
Chatopadhyay, K., Adhikari, U., Lerouge, P., and Ray, B. (2007a). Polysaccharides from Caulerpa racemosa: Purification and structural features. Carbohydr. Polym. 68, 407–415. doi: 10.1016/j.carbpol.2006.12.010
Chatopadhyay, K., Mandal, P., Lerouge, P., Driouich, A., Ghosal, P., and Ray, B. (2007b). Sulphated polysaccharides from Indian samples of Enteromorpha compressa (Ulvales, Chlorophyta): Isolation and structural features. Food Chem. 104, 928–935. doi: 10.1016/j.foodchem.2006.12.048
Chaves Filho, G. P., de Sousa, A. F. G., Viana, R. L. S., Rocha, H. A. O., Batistuzo de Medeiros, S. R., and Moreira, S. M. G. (2019). Osteogenic activity of non-genotoxic sulfated polysaccharides from the green seaweed Codiurn sertularioides. Algal Res. 14, 101546. doi: 10.1016/j.algal.2019.101546
Ciancia, M., Quintana, I., Cervellini, G., Kasulin, L., and Díaz, A., Estevez, J. M., et al. (2007). Polysaccharides from the green seaweeds Codium fragile and C. verinlaria with controversial effects on hemostasis. Int. J. Biol. Macromol. 41, 641–649. doi: 10.1016/j.jbiomac.2007.08.007
Ciancia, M., Quintana, I., and Cerezo, A. S. (2010). Overview of anticoagulant activity of sulfated polysaccharides from seaweeds in relation to their structures, focusing on those of green seaweeds. Curr. Med. Chem. 17, 2503–2529. doi: 10.2174/092986710791356069
Venkata Rao, P., Prasada Rao, N.V.S.A.V., and Sri Ramana, K. (1991). Structural features of the sulphated polysaccharide from a green seaweed, *Spongomorpha indica*. *Phytochemistry* 30, 1183–1186. doi: 10.1016/0031-9422(90)95199-9

Verbruggen, H., Ashworth, M., LoDuca, S. T., Vlaeminck, C., Coquyt, E., Sauvage, T., et al. (2009). A multi-locus time-calibrated phylogeny of the siphonous green algae. *Mol. Phylogenet. Evol.* 50, 642–653. doi: 10.1016/j.ympev.2008.12.018

Wang, X., Zhang, Z., Yao, Z., Zhao, M., and Qi, H. (2013). Sulfation, anticoagulant and antioxidant activities of polysaccharide from green algae *Enteromorpha linza*. *Int. J. Biol. Macromol.* 58, 225–230. doi: 10.1016/j.ijbiomac.2012.07.077

Wutz, M., and Zetsche, K. (1976). *Zur Biochemie und Regulation des Heteromorphen Generationswechsels der Grünalge*. *Planta* 129, 211–216. doi: 10.1007/BF00398259

Yu, Y., Li, Y., Du, C., Mou, H., and Wang, P. (2017). Compositional and structural characteristics of sulfated polysaccharide from *Enteromorpha prolifera*. *Carbohydr. Polym.* 165, 221–228. doi: 10.1016/j.carbpol.2017.02.011

Zhang, M., Zhao, M., Qing, Y., Luo, Y., Xia, G., and Li, Y. (2020). Study on immunostimulatory activity and extraction process optimization of polysaccharides from *Caulerpa lentillifera*. *Int. J. Biol. Macromol.* 143, 677–684. doi: 10.1016/j.ijbiomac.2019.10.042

Zulkifly, S. B., Graham, J. M., Young, E. B., Mayer, R. J., Piotrowski, M. J., Smith, L., et al. (2013). The genus *Cladophora* Kütsing (Ulvophyceae) as globally distributed ecological engineer. *J. Phycol.* 49, 1–17. doi: 10.1111/jpy.12025

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Ciancia, Fernández and Leliaert. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.