J.Black Sea/Mediterranean Environment
Vol.20, No. 3: 253-263 (2014)

RESEARCH ARTICLE

Anatomical features of *Cymodocea nodosa* growing in Montenegro (Adriatic Sea)

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Abstract

Compared to morphological studies of *Cymodocea nodosa* (Ucria) Asch. the anatomy of this protected seagrass was poorly studied, and the anatomical features of the *C. nodosa* leaf were not described for the South-East Adriatic Sea at all. This study was performed on three *C. nodosa* meadows in the Bay of Boka Kotorska (Montenegro, Adriatic Sea) and anatomical characteristics of sheaths and leaf blades are presented. On the cross-section of the sheaths, dorsal epidermal cells are smaller compared to the ventral epidermis, while on the leaf blade epidermis, there are no significant differences between the two sides. The mesophyll is situated below the epidermis. These cells are positioned around large air lacunaes, thus the mesophyll could be also called aerenchyma tissue. Vascular bundles are reduced compared to the vascular plants and always seven vascular bundles were counted (in the sheaths and leaf blade). Between the epidermis and mesophyllum, especially close to the vascular bundles and on the edges of the leaf, mechanical tissue is situated. Many tannin cells were observed. More detailed analysis would be necessary to explain differences and to compare them with the populations from other parts of the Mediterranean basin.

Keywords: Seagrass, *Cymodocea nodosa*, anatomy, Adriatic, Montenegro

Introduction

Seagrass meadows are one of the most productive coastal communities, playing key ecological roles in lagoons, estuarine and marine ecosystems by providing food and shelter for many important organisms, stabilising sediments and regulating nutrient cycles (Larkum *et al*. 1989; Short *et al*. 2007; Garrido *et al*. 2013). However, they are rapidly declining across the globe, with rates increasing from a median of 0.9% per year before 1940 to 7% per year after 1990; because of these high loss rates, seagrass meadows are among the most threatened ecosystems on earth (Waycott *et al*. 2009). Concern for the protection of *Cymodocea nodosa* (Ucria) Asch. and other seagrasses could be assessed through numerous national and international legislations, where these species are listed as protected or endangered (Službeni list 76/06; Barcelona Convention 1976; Bern Convention 1979; Council Directive 92/43/CEE).

Until now, for the coast of Montenegro (South-East Adriatic Sea), there have been several reports on the distribution of *C. nodosa* meadows, however, this is not sufficient to provide knowledge of distribution, monitoring and protection of this endangered seagrass (Stjepčević and Parenzan 1980; Mačić and Pal 2001; Mačić 2014). For the Mediterranean area, there are many ecological studies, as well as projects for mapping, monitoring and protecting *C. nodosa* meadows (Figure 1), although anatomical features of this seagrass and their variations are less known (Cecchereli and Cinelli 1990; Peduzzi and Vukovič 1990; Reyes and Sanson 1994; Rismondo *et al.* 1997; Mazzela *et al.* 1998; Garrido *et al.* 2013). The aim of this study is to describe the anatomical features of *C. nodosa* leaves collected from tBoka Kotorska Bay (Montenegro, Adriatic Sea) to contribute to the knowledge of this protected and endangered plant.

Figure 1. *Cymodocea nodosa* meadow (Kotor, Montenegro)

Materials and methods

The plant samples were collected by SCUBA diving in 3 locations within Boka Kotorska Bay (Kotor, Tivat and Herceg Novi) (Figure 2). In each location, 10 erect shoots of *Cymodocea nodosa* were collected at 3m depth. Furthermore, 10 shoots were collected from the deepest parts of the meadows: for Kotor, this was at 4m depth, for Tivat, at 4m depth and for Herceg Novi, at 5m depth. The anatomical features of *C. nodosa* were examined on the transverse section of adult sheaths and the leaf blade. These transverse sections of sheaths and leaf blade were made with a cryomicrotome and cutting was performed 10-20mm from the base for sheaths and for the leaf blade. Treatment with Sudan III was used to determine lipids, while tannin cells were coloured by safranin (Švob 1974). Analysis of variance (ANOVA) was performed, and factors were represented by the stations and depth.

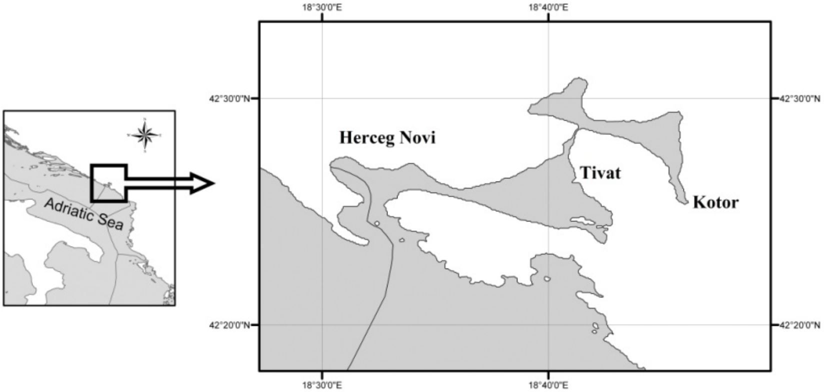

Figure 2. Location of sampling area - Boka Kotorska Bay (Montenegro)

Results and Discussion

The cross-sections of the sheaths have a characteristic shape of a letter "C", because of two lateral flaps folded over the younger leaves (Figure 3(A)). In the young sheaths, these flaps are overlapping and represent 90-118% of the overall breadth of the sheaths. As the sheaths grow, these lateral parts separate from each other until they are completely separated in the upper parts. In older sheaths, these flaps are only partially folded over the young leaves; practically speaking, they are protecting the most sensitive parts of the younger leaves, which is also the case with some other aquatic plants (Reyes and Sanson 1994).

Epidermis cells are polygonal, tightly connected and elongated in the direction of growth. As it is typical in aquatic plants, *C. nodosa* leaves have no stomata and the leaf surface is covered by a thin cuticle layer (Kuo and den Hartog 2006). On the cross-section of the sheaths, dorsal epidermal cells are smaller compared to the ventral epidermis, while on the leaf blade epidermis, there are no significant differences between the two sides (Table 1). Furthermore, the dorsal cells had a thicker cell wall, which indicated an adaptation to provide a better protection from the external environmental impacts. Another adaptation of aquatic plants to the reduced amount of light, and also to the easier exchange of gases, is a very intensive metabolism in the epidermis layer, represented by the highest concentration of chloroplast and many other organelles (Kojić 1984; Papenbrock 2012).

The mesophyll tissue is situated below the epidermis. These cells have a thin cell wall and a smaller nuguresmber of chloroplast and other organelles, compared to the epidermis. The mesophyll is not differentiated in either the palisade or the spongy layer but, according to the dimensions, two type of cells could be distinguished (Figures 4 and 5), and their dimensions are presented in Table 1. These cells are positioned around large air lacunaes, so the mesophyll could be also called aerenchyma tissue. A large proportion of leaf tissue belongs to the air lacunae, which is expected in relation to the habitats where *C. nodosa* grow. It is very well known that aerenchyma is an adaptation of the aquatic plants to the lack of oxygen in the deeper layers of the water (Terrados *et al.* 1999; Kuo and den Hartog 2006). *C. nodosa* sometimes populates sandy and muddy areas with a great amount of organic matter (Molinier and Picard 1952; Peduzi and Vukovič 1990). Decomposition of these materials cause a deficit of oxygen in the sediment and also in the water close to the bottom. Therefore, the plant responds by storing oxygen produced in the process of photosynthesis (Gibson 1984). In the case of an oxygen deficit, the gas is defunding to the organs with the smallest concentration of oxygen; thanks to these processes, *C. nodosa* is able to survive anaerobic conditions if they are not of longer character (Terrados *et al.* 1999). Beside this very important role, the function of air lacunae is also to allow a vertical position of the long leaves. In that way, the plant avoids deposition of the sediments and other material on the leaves, and is also capable of using the maximum quantity of the light (Kojić 1984).

Vascular bundles are located in the mesophyll tissue; at all three locations and at all three depths, seven vascular bundles were counted in the sheaths and leaf blade. All of them are reduced compared to the vascular plants (Kojić 1984; Kuo and den Hartog 2006), and the central one is slightly bigger than the others. Xylem cells are reduced and often completely absent.

Between the epidermis and mesophyllum, especially close to the vascular bundles and on the edges of the leaf, mechanical tissue is situated. Bunches of these very elongated cells with a thick cell wall are grouped in fibre bundles (Figures 4 and 5). The number of observed sclerenchyma bundles in sheaths and leaf blades were different across the three investigated areas (Table 2). Furthermore, there was a significant difference between samples from Tivat and the other two locations in the same depth, and also some differences between samples from different depths (Tables 3A and 3B). The highest number of fibre bundles was observed in Kotor.

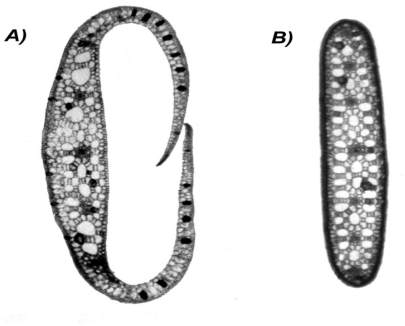

Figure 3. Cross-section: A) sheaths; B) leaf blade

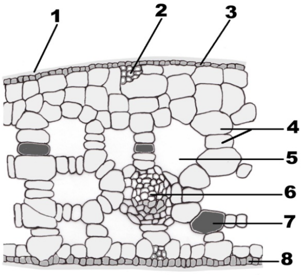

Figure 4. Cross-section of sheaths: 1. Cuticula; 2. Sclerenchyma cells; 3. Dorsal epidermis; 4. Mesophyll; 5. Air lacunae; 6. Vascular bundles; 7. Tannin cell and 8. Ventral epidermis

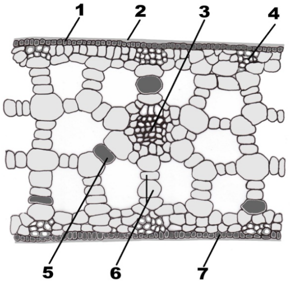

Figure 5. Cross-section of leaf blade: 1. Dorsal epidermis; 2. Cuticula; 3. Vascular bundles; 4. Sclerenchyma; 5. Tannin cell; 6. Mesophyll and 7. Ventral epidermis

Table 1. Dimensions of *Cymodocea nodosa* epidermis and mesophyll cells

Leaf Part	Tissue	Dimensions in µm	
		height	width
Sheaths	Dorsal epidermis	10-16	6-12
	Ventral epidermis	12-18	10-16
	Mesophyll bigger cells	36-63	29-47
	Mesophyll smaller cells	19-33	33-46
Blade	Epidermis	9-18	9-18
	Mesophyll bigger cells	33-43	51-61
	Mesophyll smaller cells	29-34	23-38

Table 2. Number of sclerenchyma bundles in *Cymodocea nodosa* sheaths and leaf blade

Location	Depth	Sheaths	Blade
Kotor	3	4.6	8
	4	4	15.4
Tivat	3	1.4	11.2
	4	2.2	10
Herceg Novi	3	3.4	8
	5	3.8	7.2

Table 3A. Levels of significance for number of sclerenchyma bundles for samples from A) same depth, (*-significant difference for $P < 0.05$, ns = not significant)

Leaf	Locations		
	Kotor-Tivat	**Kotor-H. Novi**	**Tivat-H. Novi**
Sheaths	*	ns	*
Blade	*	ns	*

Table 3B. Levels of significance for number of Sclerenchyma bundles for samples from different depth (*-significant difference for $P < 0.05$, ns = not significant)

Leaf	Depth		
	3m-4m	3m-5m	4m-5m
Sheaths	ns	ns	ns
Blade	*	ns	*

In the same location, the highest width of leaves' cross-sections was also measured (Tables 4 and 5). Thus we can assume that the bigger number of fibre bundles on the same location could be in relation with that, although more detailed analysis is needed in order to make a definitive conclusion.

In the mesophyllum of *C. nodosa,* many tannin cells were observed (Table 4). They are of different shapes and dimensions, usually elongated and with a big vacuole. In the sheaths, they are more often in the lateral flaps and, compared to the leaf blades, they are more numerous in the sheaths. For seagrass *Posidonia oceanica,* Pergent (1990) reported a significant increase in the number of tannin cells with the age of the rhizome, but some authors (Dumay *et al.* 2004; Pergent *et al.* 2008) reported that the production of secondary metabolites and the number of tannin cells largely increased when the degree of interaction with invasive algae *Caulerpa taxifolia* increased. Although allelopathy (a chemical process that a plant uses to keep other plants from growing too close to it) is well known in the terrestrial environment, it is not very well studied in marine plants (Dumay *et al.* 2004). The positive reaction of tannin cells with reagent Sudan III indicates the presence of lipids, but we have no precise data on the content. In general, the tannin cells contain different metabolites like proteins, lipids, and phenols, and they play an important role in the protection of plants against predators, competitors and pathogens (Ayensu 1972; Simpson 1997; Agostini *et al.* 1998). Furthermore, different metabolites from seagrasses are a source of compounds for potential use in pharmaceuticals and other purposes (Bhosale *et al.* 2002; Anselmi *et al.* 2004; Haznedaroglu and Zeybek 2007). It is well known that morphological characteristics of *C. nodosa* are variable corresponding the different environmental conditions, especially the fluxes in the concentrations of organic matter (Diliberto 1989; Pedersen *et al.* 1997; Kraemer and Mazzella 1999; Sghaier *et al.* 2011). The concentrations of organic matter are most likely the reason for some of the variations observed in the dimensions of anatomical features.

However, a more detailed analysis would be necessary to explain these differences and to compare them with the populations from other parts of the Mediterranean basin.

Table 4. Dimensions of some anatomical parameters for *C. nodosa* sheaths

Parameter	**Location and Depths**					
	Kotor		**Tivat**		**Herceg Novi**	
	3m	**4m**	**3m**	**4m**	**3m**	**5m**
Width (µm)						
Mean	2234	2124	1870	2005	2121	1861
SE	80	67	46	80	27	30
Min	2082	1951	1768	1834	2043	1807
Max	2489	2318	2043	2213	2213	1965
Thickness (µm)						
Mean	314	332	319	327	332	286
SE	12	5	5	17	9	7
Min	275	327	301	291	301	275
Max	353	353	327	373	353	314

Tablo 4. Continued

Parameter	Location and Depths					
	Kotor		**Tivat**		**Herceg Novi**	
	3m	**4m**	**3m**	**4m**	**3m**	**5m**
Number of vascular bundles						
Mean	7	7	7	7	7	7
SE	0	0	0	0	0	0
Min	7	7	7	7	7	7
Max	7	7	7	7	7	7
Main vascular bundle length (µm)						
Mean	78,85	73,27	75,59	80,50	82,30	69,66
SE	3,95	3,09	1,56	4,33	3,22	2,16
Min	64,50	67,08	72,24	67,08	73,53	61,92
Max	86,43	83,85	79,98	89,01	92,88	74,82
Main vascular bundle width (µm)						
Mean	61,81	58,31	68,37	65,27	66,56	60,63
SE	2,01	1,18	3,24	3,40	1,12	1,78
Min	55,47	55,47	59,34	55,47	63,21	54,18
Max	67,08	60,63	76,11	72,24	69,66	64,50

Table 5. Dimensions of some anatomical parameters for *Cymodocea nodosa* leaf blade

	Kotor		**Tivat**		**Herceg Novi**	
	3 m	4 m	3 m	4 m	3 m	5 m
Width (µm)						
Mean	1773	1666	1658	1724	1684	1726
SE	53	48	49	70	97	32
Min	1664	1585	1572	1559	1467	1651
Max	1926	1847	1845	1913	2044	1847
Thickness (µm)						
Mean	322	340	318	306	311	288
SE	16	13	13	21	27	7
Min	288	288	288	223	236	275
Max	360	367	354	341	406	314
Number of vascular bundles						
Mean	7	7	7	7	7	7
SE	0	0	0	0	0	0
Min	7	7	7	7	7	7
Max	7	7	7	7	7	7
Main vascular bundle length (µm)						
Men	74,05	90,30	79,68	77,14	82,04	74,30
SE	2,99	5,49	1,86	2,36	4,52	3,07
Min	65,79	77,40	75,38	68,37	70,95	67,08
Max	81,27	103,20	85,98	81,27	98,04	83,85
Main vascular bundle width (µm)						
Mean	62,44	67,08	68,09	64,24	66,56	60,11
SE	2,26	5,11	2,25	4,15	4,21	1,66
Min	54,18	54,18	61,92	52,89	54,18	55,47
Max	67,08	79,98	75,30	76,11	78,69	64,50

Table 6. Range of numbers for tannin cells on cross-sections of *C. nodosa* sheaths and leaf blades

Location	**Herceg Novi**		**Tivat**		**Kotor**	
Depth	**3m**	**5m**	**3m**	**4m**	**3m**	**4m**
Blade	3 - 16	2 - 18	2 – 11	3 - 13	6 - 12	3 - 14
Sheath	6 - 21	3 - 28	7 – 16	7 - 19	17 - 23	15 - 38

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Received:13.08.2014
Accepted:26.09.2014