Acta Adriatica 64 (2023): 33-44
https://doi.org/10.32582/aa.64.1.9
ORIGINAL ARTICLE

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Morphological and reproductive phenology of *Cystoseira foeniculacea* f. *tenuiramosa* (Phaeophyceae, Fucales) from the lagoon of Strunjan (Gulf of Trieste, northern Adriatic)

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Abstract: The results of a study on morphological features and reproductive phenology of the brown alga *Cystoseira foeniculacea* f. *tenuiramosa* (Ercegović) A. Gómez Garreta, M.C. Barceló, M.A. Ribera & J. Rull Lluch from the marine lagoon of Strunjan (Gulf of Trieste), are reported. Based on monthly samplings carried out during one year period, it was observed that this species shows considerable seasonal morphological variations. The Strunjan marine lagoon appears to be the only location of the Slovenian coast where population of this taxon occur. Based on the high associated biodiversity, we propose the inclusion of this population in the conservation management plans in the area.
Keywords: *Cystoseira foeniculacea f. tenuiramosa*; morphological features; reproductive phenology; marine lagoon Strunjan; northern Adriatic

Sažetak: MORFOLOŠKA I REPRODUKTIVNA FENOLOGIJA *CYSTOSEIRA FOENICULACEA* F. *TENUIRAMOSA* (PHAEOPHYCEAE, FUCALES) U STRUNJANSKOJ LAGUNI (TRŠĆANSKI ZALJEV, SJEVERNI JADRAN). Prikazani su rezultati istraživanja morfoloških značajki i reproduktivne fenologije smeđe alge *Cystoseira foeniculacea* f. *tenuiramosa* (Ercegović) A. Gómez Garreta, M.C. Barceló, M.A. Ribera & J. Rull Lluch u morskoj laguni Strunjan (Tršćanski zaljev). Na temelju mjesečnih uzorkovanja, provedenih tijekom jedne godine, utvrđeno je kako ova vrsta pokazuje značajne sezonske morfološke varijacije. Strunjanska morska laguna vjerojatno je jedina lokacija na slovenskoj obali gdje obitava populacija ove svojte. Na temelju velike povezane bioraznolikosti, predlažemo uključivanje ove populacije u planove upravljanja očuvanja na tom području.
Ključne riječi: *Cystoseira foeniculacea* f. *tenuiramosa*; morfološka obilježja; reproduktivna fenologija; morska laguna Strunjan; sjeverni Jadran

INTRODUCTION

The taxonomy of *Cystoseira* C. Agardh has been recently revised by Orellana *et al.* (2019) who, based on phylogenetic analyses, concluded that the genus was polyphyletic and resolved in three distinct clades: *Cystoseira s.s.* to which *C. foeniculacea* (Linnaeus) Greville and related *taxa* should be referred to, and *Carpodesmia* Greville and *Treptachanta* Kützing, the last two predated by *Gongolaria* Boehmer and *Ericaria* Stackhouse (see Molinari Novoa and Guiry, 2020).

Species of *Cystoseira* complex are often the dominant species on the rocky bottom in the Mediterranean Sea and provide a suitable habitat for many additional species; thus, it is important to understand the distribution of biocenosis characterized by these algae. Moreover, it's noteworthy that UNEP/RAC-SPA (2009) proposed protection for the species of this complex, so that countries adhering to the Barcelona Convention are obliged to protect them.

At present, a total of 35 *taxa* at specific and intraspecific level of *Cystoseira s.s.* are recognized (Guiry and Guiry, 2022) most of them occurring in the Adriatic Sea (Antolić *et al*., 2010; Cormaci *et al*., 2012; Thibaut *et al*., 2015). According to many published data, species of the *Cystoseira* complex are sensitive to the anthropogenic impact, and a decrease in their populations was observed, especially in urban areas, due to the transformation or destruction of coastal marine habitats, environmental pollution, and climate change (Thibaut *et al*., 2005, 2009; Benedetti *et al*., 2001; Mačić, 2007; Mačić *et al*., 2010). The disappearance of *Cystoseira* complex species from shallow rocky bottoms is considered an indication of severe environmental degradation (Iveša *et al*., 2016). Over the past ten years, negative changes in macrophyte spatial and seasonal diversity and a loss in the cover of canopy-forming taxa (especially *Cystoseira* complex spp.) have been detected in Slovenian coastal waters as well (Orlando-Bonaca and Rotter, 2018).

Cystoseira foeniculacea f. *tenuiramosa* (Ercegović) A. Gómez Garreta, M.C. Barceló, M.A. Ribera and J.

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Received: 18 April 2022, accepted: 23 March 2023
ISSN: 0001-5113, eISSN: 1846-0453

Rull Lluch was described from samples in Croatia by Ercegović (1952), as *Cystoseira discors* (Linnaeus) C. Agardh f. *tenuiramosa*. Following description, taxon was widely reported from the Adriatic Sea [Pignatti and Giaccone (1967), as *C. discors* f. *tenuiramosa* Ercegović, from the Gulf of Trieste; Giaccone (1978), as *C. ercegovicii* f. *tenuiramosa* Ercegović from the Gulf of Trieste; Amico *et al*. (1985), as *C. ercegovicii* Giaccone f. *tenuiramosa* (Ercegović) Giaccone (invalidly published name), from the Gulf of Trieste; Ercegović (1952), as *C. discors* (Linnaeus) C. Agardh f. *tenuiramosa* from mid-eastern Adriatic; Rizzi Longo (1972), as *C. discors* f. *tenuiramosa* Ercegović (Tremiti) from mid-western Adriatic; Giaccone and Bruni (1971) as *Cystoseira ercegovicii* (misapplied name) from Gargano (mid-western Adriatic); Mačić *et al*., (2010), as *C. foeniculacea* f. *tenuiramosa* (Ercegović) A. Gómez Garreta *et al*. from Montenegro (southeastern Adriatic); Giaccone (1970), as *C. discors* f. *tenuiramosa* Ercegović from Puglia (southwestern Adriatic)] and from lagoon of Strunjan by Battelli and Gregorič (2020). *C. foeniculacea* f. *tenuiramosa* lives on both rocky and soft bottoms near to the surface, mostly in sheltered and well-lit natural as well as artificial areas (bays and small marinas) (Cormaci *et al*., 2012; Bouafif *et al.,* 2016).

Thalli of this taxon were found in the Stjuža marine lagoon of Strunjan mainly as epiphyte on ball-like form of the red alga *Rytiphlaea tinctoria* (Clemente) C. Agardh. But, during the winter/spring period we also observed the presence of a high density of thalli free floating in the north-western part of the lagoon. These thalli were completely detached from the branches of *R. tinctoria*, due to tidal currents flowing during the tidal switch, but especially to the force of the winds that manage to tear the thalli of *Cystoseira* from those of the *Rytiphlaea* on which they are attached.

In this paper, we present the results of a study on morphological features and reproductive phenology of the brown alga *C. foeniculacea* f. *tenuiramosa* from the marine lagoon of Strunjan (Gulf of Trieste), aiming at providing useful information for its conservation.

MATERIALS AND METHODS

Research area

The Marine lagoon of Strunjan, is in the eastern part of the Strunjan Bay, Slovenian coast (45º53'07'' N; 13º60'20'' W; Fig. 1). It has an area of about 10 hectares divided into two sub-basins: a larger northern main Stjuža lagoon (LS) and a smaller south-western flowing lagoon (FL) (Fig. 1B). It is mostly a very shallow lagoon of about 0.5–1 m depth. Because of the substantial water exchange of the Stjuža lagoon with the Strunjan Bay, salinity, oxygen concentrations and thermal conditions in the lagoon are similar to that of the Bay, although the lagoon receives some freshwater inputs through small channels from agricultural areas (Vrišer, 2002). The substrate of the entire study area is characterized by soft sediment containing snail shells and organic remains that primary come from seagrasses and their rhizomes (Vrišer, 2002; Šmuc, 2020). The average tidal amplitude during the 1961-2020 period was 67 cm, with a high tide of 35 cm above the mean sea level and a low tide of 31 cm below the mean sea level (ARSO – Slovenian Environment Agency, 2022).

The lagoon is an important wetland site characterized by meadows that predominantly consist of the sea-grasses *Cymodocea nodosa* (Ucria) Ascherson and *Zostera noltei* Hornemann, which are arranged in a mosaic-like pattern, hosting a diverse lagoon fish fauna and demersal invertebrates (Avčin *et al*., 1973; Šajna and Kaligarič, 2005; Lipej *et al*., 2019). Recently a total of 15 macroalgal taxa were recorded either attached to the substrate or free floating (Avčin *et al.,* 1973; Lipej *et al.,* 2019; Battelli and Gregorič, 2020; Battelli and Catra, 2021; Battelli and Lielart, 2021). Today it is an important part of the Strunjan Stjuža Nature Reserve, which falls within the Natura 2000 network, whose main objective is the conservation of biodiversity.

Sampling procedure and data analysis

Cystoseira foeniculacea f. *tenuiramosa* was detected in the Stjuža lagoon of Strunjan on two different

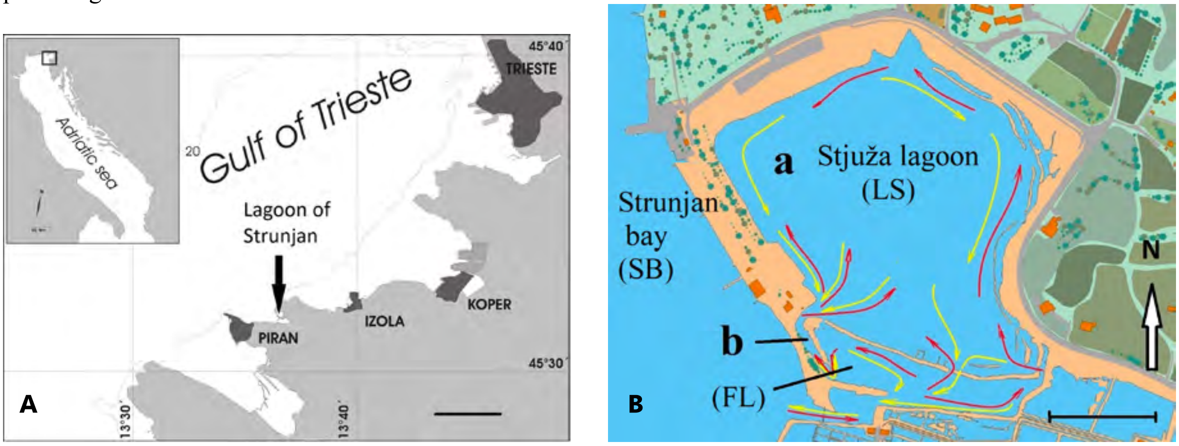

Fig. 1. Map of the study area (**A**); Map of Stjuža Lagoon of Strunjan (LS) with sampling sites (a) and (b), flowing lagoon (FL), and the direction of the currents of the sea water during tide. The yellow arrows indicate the output flow and the red arrows the entry flow (**B**). Scale bar A. 5 km; B. 100 m.

substrates: as an epiphyte on the red alga *Rytiphlaea tinctoria* balls mainly at site "a" and in very small quantities on small pebbles in the channel at site "b" in the southwestern part of the lagoon (Fig. 1B). For our study, all specimens were collected at site "a" in the western part of the Stjuža Lagoon, as this taxon is more abundant there than in the other parts of the lagoon. The observations were made only in the areas along the margins of the lagoon, as the other areas, especially the central ones, were not accessible. Based on the observations, it was possible to determine that the distribution of the population of *C. foeniculacea* f. *tenuiramosa* in the Strunjan lagoon is spatially very different. In fact, it was dense in the areas along the margin of the lagoon in the north-western and western parts, at site "a", while it was very rare or completely absent in the other areas.

Ten thalli of *Cystoseira foeniculacea* f. *tenuiramosa* were randomly collected from the site "a" of the Stjuža lagoon (SL) of Strunjan every month for one year (from March 2021 to February 2022). At the same time, the most abundant other unattached macroalgal species, accompanying the sampled *Cystoseira* thalli, were visually assessed and collected. All these fresh specimens were collected by hand at the survey site "a" and immediately placed in plastic bags with water, and then transported to the Laboratory of the Faculty of Mathematics, Natural Sciences and Information Technologies (FAMNIT) of Koper, for further observations. Upon further analysis, each *Cystoseira* thalli was examined for epiphytes that were separated and identified from the host plant. Some collected specimens of *Cystoseira* were dried, pressed, and preserved in the personal herbarium of one of the authors (C.B.).

The algal material collected was carefully sorted and examined using an Olympus SZ61 stereo microscope with a XC50 digital camera for morphological observations and measurements. The following measures were taken: thallus length, from the basal part to the apex of the thallus; length and diameter of main axes; length and diameter of primary axes. The reproductive phenology was also examined, describing the presence and the position of conceptacles in different thalli. The sizes of the ostioles of the conceptacles, were measured. In addition, the sizes of the oogonia were measured and the position of the oogonia and antheridia, was described. The monthly averages of all the measurements made were calculated and, on the basis of these values, graphs were constructed to better highlight the phenological variations of this taxon over the year. The presence of receptacles, their morphology and their degree of maturity were also detected.

Salinity, pH, oxygen content (O_2), water temperature and redox potential were measured in the Stjuža lagoon and the Strunjan bay, with a Multiparameter Waterproof Meter Hanna HI98194. The collected algal material (the studied taxon, epiphytes on it, and other most abundant unattached macroalgae present near the collected samples), was identified to the species level whenever possible and to the genus level when diacritical features were lacking. The main resources used for species identification included Maggs and Hommersand (1993), Bressan and Babbini (2003), and Cormaci *et al*. (2017, 2020, 2021) for Rhodophyta; Phillips and De Clerck (2005), Brodie *et al*. (2007), Sfriso (2010) and Cormaci *et al*. (2014) for Chlorophyta; Sfriso (2011) and Cormaci *et al*. (2012) for Ochrophyta. For the nomenclature we followed Guiry and Guiry (2023) and Worms Editorial Board (2022).

RESULTS

Cystoseira foeniculacea f. *tenuiramosa* was identified based on morphological characteristics (Fig. 2A) (details below). All the studied specimens were exclusively epiphyte on the aegagropilous *R. tinctoria* (Fig. 2B). Only a small number of thalli of *C. foeniculacea* f. *tenuiramosa* were found attached on pebbles as shown in Fig. 2C.

Morphological features of the thallus

In general, the collected specimens of *C. foeniculacea f. tenuiramosa* were erect, caespitose, with the older parts of the thallus dark-brown, and the younger ones from olive-green to brown-yellow in colour (Fig. 2A, 2B). Being epiphytic on the red alga *Rytiphlaea tinctoria*, the thalli were attached to this alga through a discoid holdfast of about 1.5 – 5.5 mm in diameter.

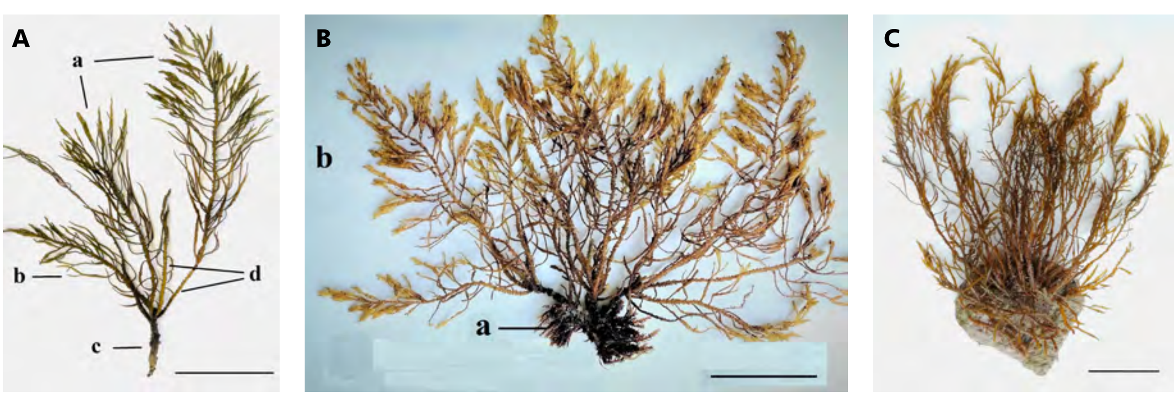

Fig. 2. General view of *Cystoseira foeniculacea* f. *tenuiramosa*: receptacles with aerocysts (a), secondary branches (b), main axes (c), primary branches (d) (**A**); *C. foeniculacea* f. *tenuiramosa* (b) as epiphyte on *Rytiphlaea tinctoria* (a) (**B**); *C. foeniculacea* f. *tenuiramosa* thalli attached on pebble (**C**). Scale bar 5 cm.

Table 1. Mean values with standard deviation of some morphological monthly measurements of *Cystoseira foeniculacea* f. *tenuiramosa* from the Stjuža lagoon of Strunjan (site "a").

Month	Thalli		Main axis				I branches			
	Length (cm)	**stdev**	**Length (cm)**	**stdev**	**diam. (mm)**	**stdev**	**Length (cm)**	**stdev**	**diam. (mm)**	**stdev**
January	27.77	3.12	3.41	1.45	3.06	0.72	18.84	3.28	1.45	0.13
February	27.17	4.31	4.19	0.93	2.92	0.57	20.70	4.08	1.60	0.20
March	23.34	3.65	3.00	0.91	3.95	0.72	15.89	1.80	1.72	0.47
April	23.23	5.79	3.90	0.83	2.91	0.55	12.74	3.06	1.62	0.34
May	15.79	5.19	4.43	2.00	3.26	0.89	8.11	2.05	1.89	0.15
June	14.16	1.06	3.66	0.85	3.53	0.66	6.73	1.34	1.91	0.31
July	14.37	2.68	4.85	1.00	3.80	0.60	6.01	1.57	2.08	0.19
August	10.82	4.17	5.50	2.06	3.45	0.51	4.81	1.89	1.63	0.18
September	8.07	2.95	2.05	1.34	2.26	1.21	4.68	1.04	1.39	0.17
October	12.11	2.16	2.79	2.34	2.54	0.79	6.14	1.43	1.50	0.39
November	15.66	4.24	2.87	1.57	2.38	0.88	8.48	1.76	1.52	0.31
December	22.48	2.97	3.49	1.60	3.10	1.08	15.45	1.62	1.42	0.20
mean	17.91	3.52	3.68	1.41	3.10	0.76	10.72	2.08	1.64	0.26
stdev	6.60	1.31	0.96	0.52	0.54	0.22	5.73	0.91	0.22	0.11

The average annual height of the thalli, calculated on the basis of the monthly averages, was 17.91 cm, from a minimum of 8.07 cm, recorded in September, to a maximum of 27.77 cm, in January (Table 1; Fig 3). In the other months, the mean height of the thalli was higher in the autumn and in the winter, than in summer. Main axes muriculate with the remnants of simple, short and spaced appendages, dark-brown in colour. The mean annual height of the main axes, was 3.68 cm, varying between a minimum of 2.05 cm, recorded in September and a maximum of 5.50 cm in August. In the other months, the mean height of the axes showed only small variations compared to the mean annual value (Table 1).

From the main axes arose about 3-4 primary branches, covered by spaced simple spinose appendages and scars, mainly cylindrical, but sometimes slightly flattened, especially at the beginning of the vegetative period, with a mean annual height of 10.72 cm. They reached the maximum value of 20.70 cm in February, significantly decreasing, to a minimum of 4.68 cm, in September. Then, branches progressively increased again their size showing a more marked growth in the period December-April.

It is interesting to note that the length of the thalli was greater in the winter months in correspondence with the maximum growth of the primary branches, while, inversely, the period in which the main axes were longer was in summer when, instead, the species has shorter primary branches (Fig. 3). Comparing the length of the thalli with the surface seawater temperature, it was noted that the increase in temperature coincided with the decrease of the length of the thalli, but coincided with the increasing trend of the length of the main axis.

The apices of the main axes were slightly prominent with respect to the insertion of the primary branches, with small simple spinose appendages (Fig. 4A). A substantial difference was found in the mean annual values of the diameter between the main axes (3.10 mm) and primary branches (1.64 mm). In both cases the values of the diameter, showed only small variations compared to the average annual values (Table 1).

A more careful observation of the morphology of the main branches showed a marked seasonal variability during the vegetative development during one year. At the beginning of the vegetative development, in summer, the thalli of *C. foeniculacea* f. *tenuiramosa*, appeared rich in short primary branches, which were entirely flattened (Fig. 4B; 5H). In autumn, on the other hand, these branches remained flattened only in the proximal part, while in the upper part they were cylindrical. Towards the end of the winter, they all became completely cylindrical, and remained so until the end of spring and the beginning of summer, when they were almost naked, without no ramifications (Fig 4C; 5G).

The collected thalli of *C. foeniculacea* f. *tenuiramosa* had cylindrical secondary branches, but often slightly flattened, while higher order branches were filiform. Foliate branches, with an evident median rib and with a toothed margin, were often present throughout the year at the base of the primary branches Fig. 4B; 4D), especially at the beginning of the vegetative period, mainly in summer and autumn (Fig. 5H; 5I). A more careful observation, regarding the seasonal variability of the secondary foliate branches, showed their presence from the beginning of the vegetative period, in summer (especially from August to September). They

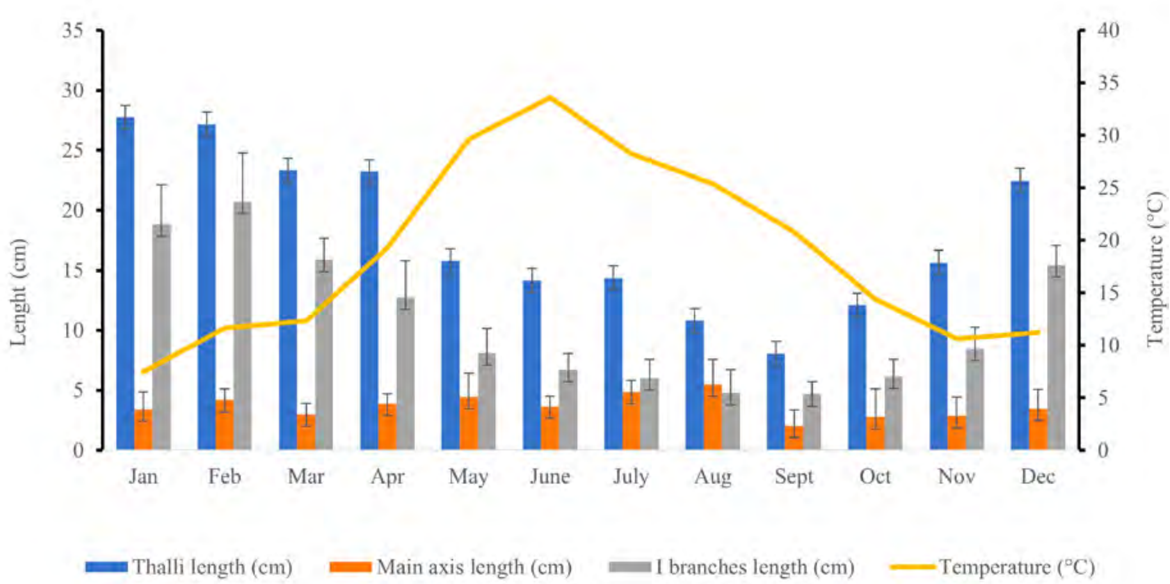

Fig. 3. Comparison among the monthly variations in dimensions of thalli, main axes, and primary branches of *Cystoseira foeniculacea* f. *tenuiramosa* (± STDEV) and mean temperature of surface seawater (°C) from the Stjuža lagoon of Strunjan (LS) (site "a").

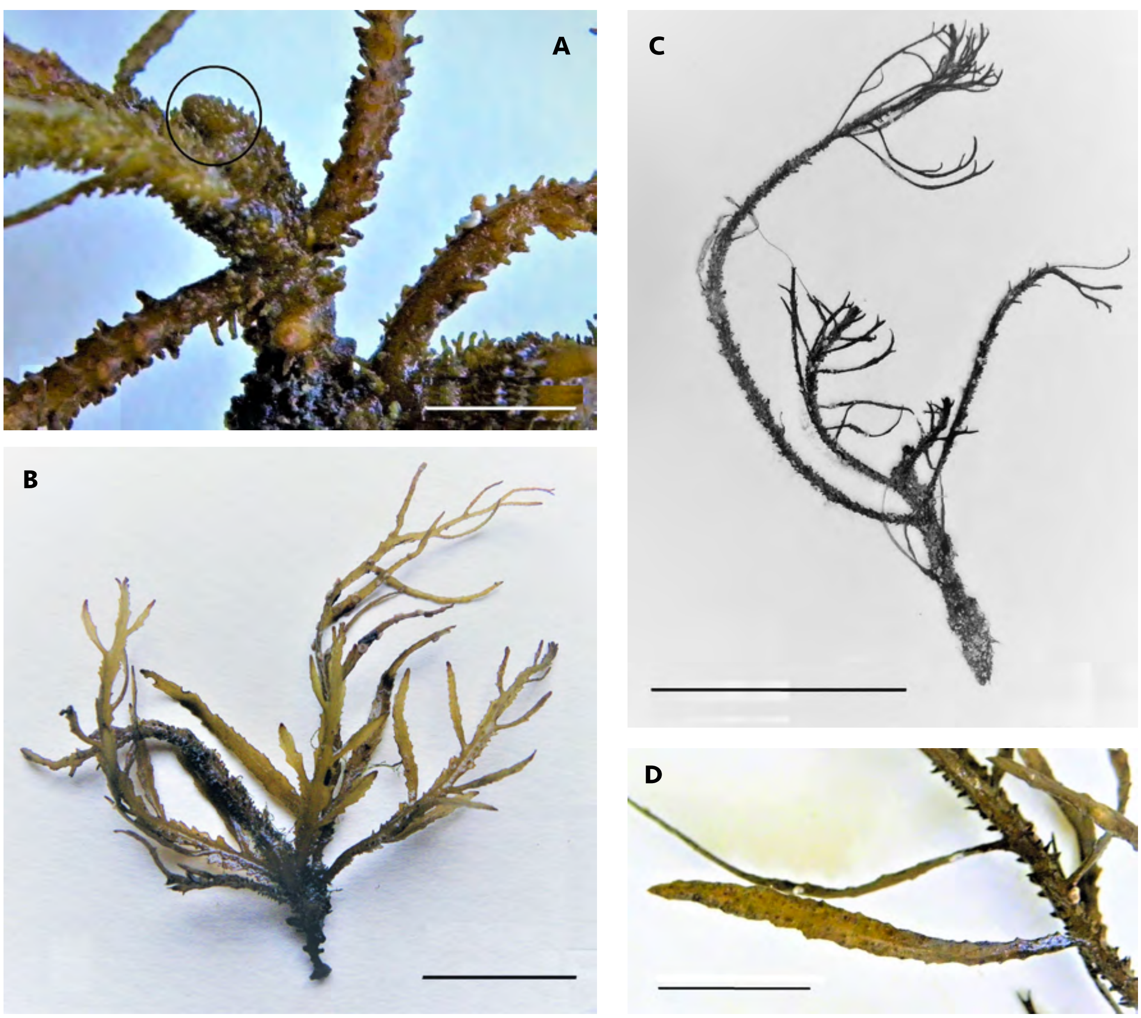

Fig. 4. Morphological features of the thalli of *Cystoseira foeniculacea* f. *tenuiramosa*. Apices of the main axes indicated with a black circle (**A**); Thallus with flattened primary branches (**B**); Naked branches without ramification (**C**); Detail of foliate branches with an evident median rib and with a toothed margin (**D**). Scale bars: A. 0.5 cm; B., C. 5 cm; D. 1 cm.

remain foliate, only in the proximal part of the primary branches, while in the upper part they were thin, cylindrical and filamentous. This situation remained static until the end of the winter (March). In spring, the secondary foliate branches became all cylindrical and filamentous on the primary branches along their entire length. They underwent a further transformation at the beginning of the summer until they were reduced to single filamentous tufts placed at the top of the primary branches (Fig. 4D; 5G).

Reproductive phenology

During the sampling period fertile thalli of *C. foeniculacea* f. *tenuiramosa*, were also found. The fusiform-cylindrical receptacles, (4.00-6.41 x 1.30-2.03 mm) (Table 2), located in the terminal part of the branchlets, distributed in general in chain series of 2-3, were simple or occasionally branched, often associated with aerocysts (Fig. 6A). They were present from November to May, but absent during the summer months (Table 2). Aerocysts oblong in chains present from November to May.

Fertile conceptacles (0.20-0.35 x 0.16-0.32 mm) (Table 2), grouped and slightly prominent, were distributed on the surface of the terminal receptacles (Fig. 6B) in the period between January and April, containing several dark brown, rounded, mature oogonia (about 0.06 mm in diameter) (Table 2) emerging from the bottom and small yellowish antheridia formed at the apices of branched filaments (Fig. 6C). During the period from May to January, they appeared emptied. The thalli of *C. foeniculacea* f. *tenuiramosa* were fertile in the winter period when the average temperature was 12.69 ºC, with a minimum of 7.49 ºC in January and a maximum of 19.29 ºC in April (Table 3).

The specimens of *C. foeniculacea* f. *tenuiramosa* were characterized by a wide range of morphological variability, depending mainly on the season, dealing with the size of the thallus, the length and the shape of

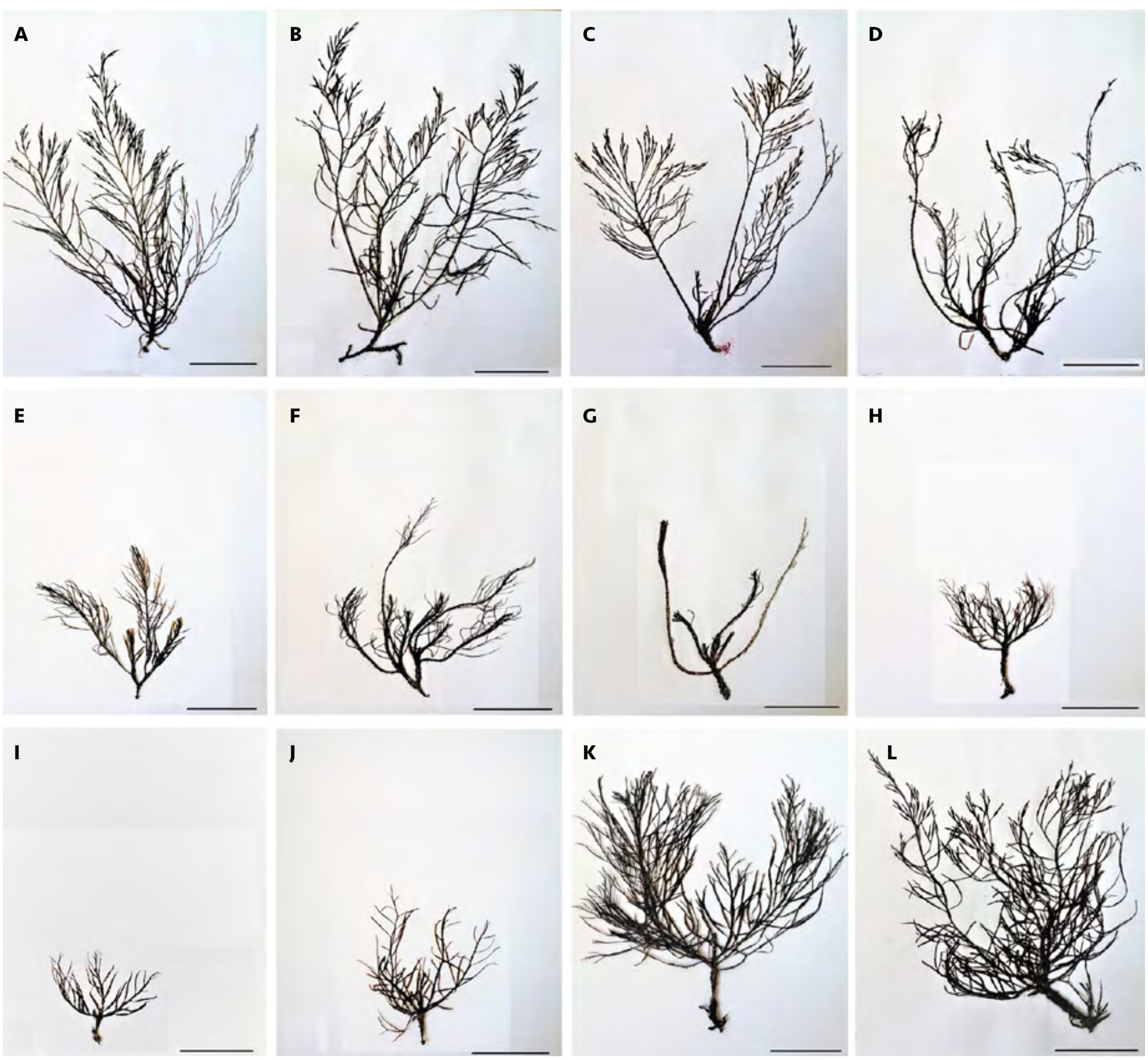

Fig. 5. Habit of the thalli of *Cystoseira foeniculacea* f. *tenuiramosa* in different month throughout the year. January (**A**); February (**B**); March (**C**); April (**D**); May (**E**); June (**F**); July (**G**); August (**H**); September (**I**); October (**J**); November (**K**) and December (**L**). Scale bars 5 cm.

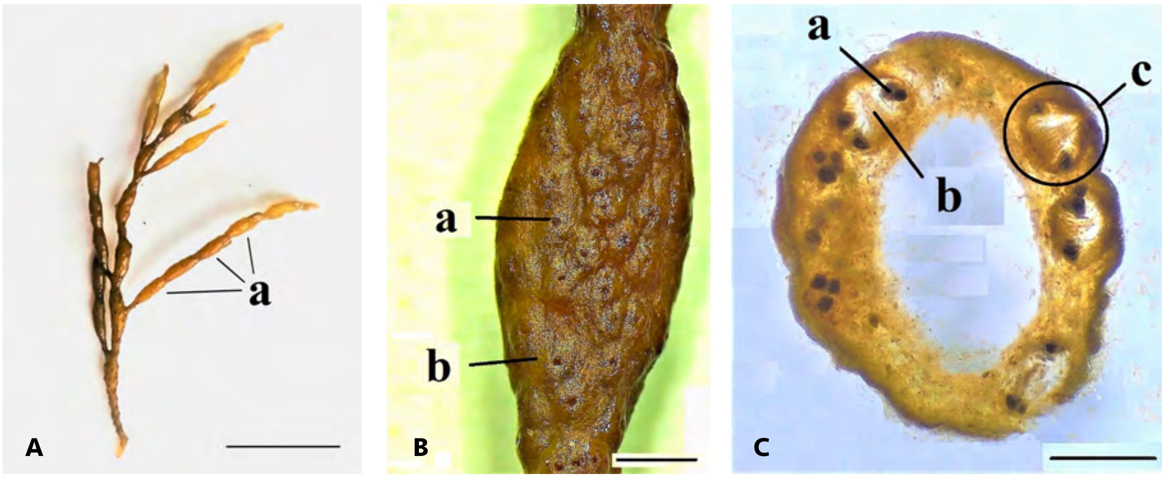

Fig. 6. Fertile thalli of *Cystoseira foeniculacea* f. *tenuiramosa*. Terminal branchlets with aerocysts in chain series (a)(**A**); Receptacle on aerocyst with ostiole (a) of the conceptacle (b) (**B**); Cross section of the thallus with conceptacles (c) containing oogonia (a) and antheridia (b) (**C**). Scale bars: A. 5 cm; B., C. 1 mm.

the main axes, of both primary and secondary branches, and the size of the receptacles (Table 2).

Our specimens basically well agree with the original diagnosis of *C. foeniculacea* f. *tenuiramosa* (Ercegović, 1952, p. 113, pl. XXVI, as C. *discors f. tenuiramosa*), and with later descriptions (Gómez-Garreta *et al*., 2001; Cormaci *et al*., 2012; Taşkin *et al*., 2012; Bouafif *et al.,* 2016). There are small differences probably due to the fact that the above authors refer to epilithic thalli living in the open sea, where the hydrodynamism of the water is more elevate, while our thalli are epiphytic and live in a very sheltered environment.

Epiphytes found on collected thalli

The most abundant epiphytes on *C. foeniculacea f. tenuiramosa* were the crustose coralline red algae *Lithophyllum cystoseirae* (Hauck) Heydrich and *Titanoderma pustulatum* (J.V. Lamouroux) Näegeli which mainly covered the older branches. Abundant were also different species of green algae among which species of the genus *Ulva* (with *Enteromorpha* like morphology) and *Cladophora*, while *Ceramium diaphanum* (Lightfoot) Roth, *Ceramium* spp., *Chondria coerulescens* (J. Agardh) Sauvageau, *C. capillaris* (Hudson) M.J.Wynne, *Polysiphonia* spp., were rarer. Many of the collected specimens were found with older branches abundantly covered with the Serpulidae *Janua heterostropha* (Montagu, 1803).

Environmental characteristics

Due to the shallow depth of the Stjuža lagoon of Strunjan, it would be expected a considerable variation of the values of physical and chemical parameters. But against expectations, during sampling period, some values from the sampling site "a" (LS), were comparable to those of the Strunjan bay (SB). For example, the highest temperature values were measured in June and the lowest in January, in both areas (Table 3). The values of pH were very similar throughout the year in both areas. Small differences were observed in the values of salinity, especially in February when the values in lagoon were lower than in the Strunjan bay. More differences were found in redox potential (Table 3). Owing to the substantial water exchange of the lagoon with the Strunjan bay, the values of the physical and chemical measurements in the lagoon are comparable to that of the bay.

Algal flora of Stjuža lagoon

In the Stjuža lagoon several other algal species, were present in both attached and unattached forms. The soft bottom was clearly unsuitable for the development of a highly diverse attached macroalgal vegetation. Due to the lack of hard substrata in the lagoon, we found the attached algae mainly on small pebbles, shells, man-made objects and seagrass rhizomes. Some species were present only in the unattached form, floating above the bottom as benthopleustophytes. Among the most abundant unattached algal species, besides the aegagropilous *R. tinctoria*, there were green algae *Ulva rigida* C. Agardh, *U. australis* Areschoug, *U. compressa* Linnaeus, *U. flexuosa* Wulfen, *U. intestinalis* Linnaeus, *U. kylinii* (Bliding) H.S. Hayden, Blomster, Maggs, P.C.Silva, Stanhope and Waaland, *Chaetomorpha linum* (O.F. Müller) Kützing, *Cladophora lehmanniana* (Lindenberg) Kützing, *C. liniformis* Kützing. The red algae comprised *Chondria capillaris* (Hudson) M.J. Wynne, *Polysiphonia scopulorum* Harvey, *Polysiphonia* sp., *Polysiphonia spinosa* (C. Agardh) J. Agardh, *Ceramium* spp., *Palisada patentiramea* (Montagne) Cassano, Sentíes, Gil-Rodríguez and M.T. Fujii, *Spyridia filamentosa* (Wulfen) Harvey, and *Alsidium corallinum* C. Agardh. Besides *C. foeniculacea f. tenuiramosa*, the brown algae were represented by only one species, *C. aurantia* Kützing, detected in the free-floating form. Two species of algae with typical ball-like forms, were observed in the lagoon: the red *R. tinctoria*, and the green *Cladophora prolifera* (Roth) Kützing.

Table 2. The sizes (in mm) of receptacles, ostiole, conceptacles and oogonia of *Cystoseira foeniculacea* f. *tenuiramosa* from the Stjuža lagoon of Strunjan (site "a").

Month	Receptacles with aerocysts/ terminal			Ostioles of conceptacles			Oogonia	Conceptacles		
	Length (mm)	Width (mm)	l/w ratio	Length (mm)	Width (mm)	l/w ratio	Width (mm)	Length (mm)	Width (mm)	l/w ratio
January	4.75	2.03	2.67	0.14	0.08	1.81	0.05	0.26	0.20	1.32
February	5.18	1.94	2.71	0.10	0.08	1.05	0.08	0.35	0.32	1.10
March	6.41	1.92	3.36	0.15	0.10	1.59	0.07	0.29	0.23	1.27
April	5.37	1.51	3.57	0.23	0.18	1.32	0.05	0.26	0.21	1.31
May	5.29	1.62	3.44	0.38	0.30	1.31	absent	0.23	0.18	1.29
June	-	-	-	0.14	0.10	1.51	absent	0.26	0.21	1.25
July	-	-	-	0.13	0.09	1.50	absent	0.23	0.20	1.18
August	-	-	-	0.12	0.09	1.32	absent	0.22	0.18	1.23
September	-	-	-	0.10	0.08	1.50	absent	0.22	0.17	1.30
October	-	-	-	0.11	0.10	1.12	absent	0.21	0.18	1.20
November	4.00	1.30	3.23	0.13	0.09	1.39	absent	0.20	0.16	1.25
December	4.84	1.82	2.68	0.13	0.09	1.58	absent	0.20	0.16	1.26
Mean	5.12	1.73	3.09	0.16	0.11	1.42	0.06	0.24	0.20	1.25
stdev	0.74	0.27	0.39	0.08	0.06	0.21	0.01	0.04	0.04	0.06

Table 3. Seasonal variation of some environmental parameters (salinity, temperature, pH, oxygen content and redox potential) from the sampling site "a" (LS) of the lagoon of Strunjan and Strunjan bay (SB). Measurements in February were not available.

	Salinity		Temperature (°C)		pH		O_2 (ppm)		Redox	
Month	**LS**	**SB**	**LS**	**SB**	**LS**	**SB**	**LS**	**SB**	**LS**	**SB**
April	34.6	35.2	19.3	16.3	8.4	8.3	10.5	11.2	35.4	36.9
May	28.0	30.3	29.6	19.6	8.8	8.3	12.6	10.6	58.8	74.8
June	30.4	28.3	33.6	29.1	8.8	8.3	9.0	7.5	105.4	72.0
July	27.9	27.4	28.2	27.5	8.7	8.3	8.7	8.4	87.3	67.5
August	31.7	30.9	25.4	25.2	8.2	8.3	7.3	8.3	44.8	19.0
September	30.0	30.7	20.8	22.1	8.3	8.4	2.4	2.5	46.4	12.6
October	28.1	29.7	14.4	17.1	8.1	8.3	11.6	10.8	32.1	41.4
November	30.1	31.2	10.7	13.5	8.2	8.2	8.3	7.6	60.7	70.6
December	24.5	26.0	11.3	11.6	7.9	8.2	8.3	8.8	26.8	34.4
January	30.7	31.1	7.5	10.3	8.1	8.3	7.3	8.6	95.2	91.5
February	28.1	35.3	11.6	10.5	8.1	8.0	-	-	16.8	48.2

DISCUSSION

On the basis of our observations and the study of the available literature, at present, along the coastal water of Slovenia, are present two forms of the species *C. foeniculacea*: *C. foeniculacea* f. *latiramosa* (Ercegović) A. Gomez Garreta *et al*., and *C. foeniculacea* f. *tenuiramosa*. *C. foeniculacea* f. *latiramosa* was reported only once from Slovenian waters, in the Strunjan Bay in the year 1972 (Avčin *et al*., 1973). Only recently, after fifteen years, this taxon was found again by Orlando-Bonaca and Trkov (2020) along the coast between Koper and Izola (Koper Bay). *C. foeniculacea* f. *tenuiramosa*, was found in the marine lagoon of Strunjan by Battelli and Gregorič (2020), mainly as epiphyte on the red alga *Rytiphlaea tinctoria*. The occurrence of the epilithic form of this species from the Gulf of Trieste (north Adriatic), was reported for the first time by Pignatti and Giaccone (1967), as *C. discors* f. *tenuiramosa*. About the occurrence of epiphytic form of this species, to date, there are no information, thus, according to the available information, the marine lagoon of Strunjan appears to be the only station of the northern Adriatic where *C. foeniculacea* f. *tenuiramosa* occurs.

During this study, populations of the above infraspecific taxon were found on two attached substrates in the lagoon of Strunjan: as epiphyte on the red alga *R. tinctoria* balls in the main Stjuža lagoon, and epilithic on small pebbles in the small canal on the south part of this lagoon. Based on the information available, studies on epiphytic form of *Cystoseira* species, are rare. However, there are cases reported in the literature of *Cystoseira* species as epiphyte on other *Cystoseira* species. Cormaci *et al*. (2012), for example, reported the case of *Cystoseira humilis* Schousboe ex Kützing as the only species of *Cystoseira* capable to live as epiphyte on others species of *Cystoseira*. An example of this is provided by Devescovi (2015) observed that, in the west Istrian coast (northern Adriatic, Croatia), *C. humilis* tended to grow as an epiphyte on another host *Cystoseira* spp. While Mačić and Svirčev (2014) reported, instead, the presence of *Gongolaria barbata* (Stackhouse) Kuntze (as *Treptacantha barbata* (Stackhouse) Orellana and Sansón), as epiphyte on the thalli of *C. crinitophylla* Ercegović, from the coast of Montenegro.

Indeed, unlike other species of the *Cystoseira* complex, the morphological and reproductive phenology of *C. foeniculacea* f. *tenuiramosa* has been poorly investigated and relatively little data on the vegetative growth and reproductive cycle, are available. The morphological characteristics of the collected thalli of *C. foeniculacea* f. *tenuiramosa* partially coincide with those reported by Ercegović (1952, as C. *discors* f. *tenuiramosa*) and later by Gómez-Garreta *et al*. (2001), Cormaci *et al*. (2012) and Taşkin *et al*. (2012), who reported: (a) primary branches cylindrical; (b) secondary and upper order branches cylindrical, filiform. Ercegović (1952) added some observations, especially on the morphology of the secondary branches which are very close to those reported in this work, as follows: (i) secondary branches of the lower part of the primary branches, flattened and foliose only in the young thalli, while in the adult thalli are only slightly flattened but not foliose; (ii) secondary branches on the upper parts of primary branches cylindrical.

About the morphology of the branches, it is interesting to note that for the species *C. humilis*, Cormaci *et al*. (2012) reported that primary branches are more flattened in the epiphytic thalli, than in the epilithic ones. This information agrees with the fact that our specimens differ from the epilithic ones, as reported in the literature, in having more flattened primary branches. This leads us to suppose that the epiphytic forms have slightly more flattened branches than the epilithic ones. Unfortunately, there are no studies dealing with this phenomenon, so further studies and verifications, are necessary. It is important to note that the above authors reported observations on epilithic specimens collected from exposed sites, where the hydrodynamism is more elevate then in lagoon. From a more careful examination it appeared that the variability of the morphological characters of the primary and secondary branches of our specimens differ slightly from those reported in the literature. In fact, the primary branches remain flattened for a long time and the secondary ones remain also foliose for a long time.

The monthly collection of *C. foeniculacea* f. *tenuiramosa* specimens during one year, also allowed us to follow the growth and development of the thalli of this species. We observed that the specimens were characterized by the development of thalli throughout all the year, even though their growth was not equally intense. The main development period, characterized by fast growth of main branches, was from mid-autumn (August – September) to mid-spring (April). While a decrease in the size of the thallus was noted from the second half of spring to the first half of summer (from May to September). In particular, the main differences were noted in the size of the thallus, the length of the main axes and primary branches, the shape and size of the secondary branches and the receptacles. The length of the thalli was greater in the winter months in correspondence with the maximum growth of the primary branches, while, inversely, the period in which the main axes were longer was in summer when, instead, the species has shorter primary branches.

As concerned the reproductive phenology, we found that the collected specimens of *C. foeniculacea* f. *tenuiramosa* were fertile in winter and spring time. Indeed, the receptacles with fertile conceptacles were present from January to April. The receptacles were associated with aerocysts, which appeared from mid-autumn to mid-spring (from November to May), while Ercegović (1952) reported the presence of aerocysts from March/April to the end of spring.

About the impact to the environment, Belegratis *et al*. (1999), for example underlined the importance of the epiphytes in marine biocoenosis. The authors pointed out the contribution of epiphytes to primary productivity, and interactions with host algal species and with other species of communities. Unfortunately, informations on the status of the lagoon environment are very rare. From literature data (Lipej *et al.* 2019) most of the macroalgal species found belong to the ESGII class, which comprised fleshy opportunistic (IIB) and filamentous sheet-like opportunistic (IIA) species both in coastal and transitional waters (Orfanidis *et al*., 2011), which means that they are not indicators of good ecological status. Lipej *et al.* (2004) reported a sporadic occurrence in the Stjuža lagoon of the brown algae *Cystoseira compressa* (Esper) Gerloff and Nizamuddin and *Fucus virsoides* J. Agardh, which are indicators of good ecological status, but we did not find those species during this study. On the other hand, the occurrence of *C. foeniculacea* f. *tenuiramosa* and *Cystoseira aurantia* Kützing reported by Battelli and Catra (2021), is certainly a positive indication of the species diversity of the lagoon. Unfortunately, up to now, there are no studies regarding the ecological conditions that could somehow explain the occurrence of epiphytic form of *C. foeniculacea* f. *tenuiramosa* in the lagoon, especially because this taxon it is not present, at the moment, in the coastal waters of Slovenia.

During our study, it was noted that in winter and in early spring (period when the *C. foeniculacea* f. *tenuiramosa* reaches the maximum development), a great mass of floating thalli of *C. foeniculacea* f. *tenuiramosa* detached from the host algae was present, probably due to the action of the winds. At the moment we have no information on the consequences that such an accumulation could have on the fauna, the algal vegetation, and on the seagrasses of lagoon. According to Belegratis *et al*. (1999), the presence of so high density of *C foeniculacea* f. *tenuiramosa* could act as a barrier to light penetration reducing the photosynthesis of hosts and could cause a population regression.

Our assumptions are based only on the observations made during the short research period and the study of the available literature, too short to draw conclusions about the consequences of this phenomenon. Thus, further investigations are desirable and necessary. Both literature data (Avčin *et al*., 1973; Vrišer, 2002; Lipej *et al*., 2019) and our measurements show that some physical and chemical parameters like salinity, thermal conditions, pH, oxygen content, and redox potential, in the Stjuža lagoon are associated with exchange with the Strunjan bay and thus, are generally similar to those of the Strunjan bay. For these reasons, we do not consider the occurrence of the aegagropilous masses of *R. tinctoria* with the epiphytic form of *C. foeniculacea* f. *tenuiramosa* as a disruptive algal bloom proliferating due to anthropogenic factors (e.g. increased nutrient levels). In our opinion, those algae do not pose a threat to the existing ecosystem that mainly consists of the seagrass's meadows of *C. nodosa* and *Z. noltei*, where this aggregation occurs.

Among the beneficial effects for the host the above authors mention the limitation to grazing by potential herbivores and the reduction of desiccation stress during low tide. This could also be the case of *C. foeniculacea* f. *tenuiramosa* as epiphyte on *R. tinctoria*. The bottom of the lagoon is very low (just 0.5-1 m depth), so during low tide they can be, for short periods, out of the water. Physical factors, such as temperature, wave exposure, rate of sedimentation, together with biological factors, phenology and architecture of algal host, might play an important role in the life of both the host and the hosted organism. So, for understanding the causes of distribution of epiphytic *Cystoseira* spp. and other macroalgal species, the complex analysis of abiotic and biotic factors should be performed, as reported also by Mačić and Svirčev (2014). Finally, we would like to underline that the thalli of *C. foeniculacea* f. *tenuiramosa*, in the Stjuža lagoon of Strunjan, occurred mainly as epiphyte on a macroalga. Such a particular behaviour seems to be the only to date reported for a *Cystoseira* species. In fact, the only other cases of epiphytism observed in *Cystoseira* species dealt with species of *Cystoseira* complex epiphytic on other species of *Cystoseira*, noted by Cormaci *et al*. (2012) and Devescovi (2015) for *C. humilis*, and Mačić and Svirčev (2014) for *G. barbata*.

CONCLUSIONS

The results lead to several important considerations:

- The morphological characteristics of the collected thalli of *C. foeniculacea* f. *tenuiramosa* well coincide with those reported in literature, but are slightly different in some features. In fact, the primary branches remain flattened and the secondary ones remain foliose for a longer time, in comparison with what reported in literature for epilithic form of this taxon.
- Results of our study on both growth and reproduction of *Cystoseira foeniculacea* f. *tenuiramosa* are particularly important for a better understanding of the abundance and distribution of the epiphytic species, and therefore also of their community structure. So, for better understanding the causes driving distribution of epiphytic *Cystoseira* species, the complex analysis of abiotic and biotic factors should be performed.
- Due to the high ecological value of *Cystoseira* complex spp., our finding is quite important, and the presence and abundance of *Cystoseira foeniculacea* f. *tenuiramosa* in the Stjuža lagoon of Strunjan should be regularly monitored in the future.

AKNOWLEDGMENTS

The authors wish to thank the staff of Public Institute Landscape Park Strunjan (Slovenia), for allowing us to carry out the research. Dr Vesna Mačić from Institute of Marine Biology of Montenegro, for the precious information regarding the epiphytes on the *Cystoseira* species. Dr Peter Glasnovič from the Faculty of Mathematics, Natural Sciences and Information Technologies (FAMNIT) of Koper, who kindly helped us during the measurements of morphometric characters of the samples. Bojana Lipej from Škocjanski zatok Nature Reserve, who helped us during the measurements of physical and chemical parameters of the lagoon. Special thanks to Professor Giovanni Furnari from the Laboratory of Phycology, Department of Biological, Geological and Environmental Sciences of the University of Catania, for his valuable advice and the taxonomic review of the manuscript. We thank the anonymous reviewers whose comments helped improve the manuscript.

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