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First report of an aegagropilous form of *Cladophora prolifera* (Cladophorales, Chlorophyta) from the lagoon of Strunjan (Gulf of Trieste, northern Adriatic)

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Abstract

The occurrence of unattached spherical (aegagropilous) populations of the green alga *Cladophora prolifera* in the marine lagoon of Strunjan (Gulf of Trieste) has been reported in this study. Species identification was based on molecular and morphological data. Further, the distribution, ecology, and morphological features of the aegagropilous populations have been delineated. The ball-shaped form of this species differs from the typical upright attached form found on open shores by the radial arrangement of the branches and the absence of cells and rhizoids with clear annular constrictions. The aegagropilous form of *C. prolifera* probably emerges from specific hydrodynamic conditions in the human-mediated environment of the marine lagoon, in combination with the morphological development of the species. The Strunjan marine lagoon appears to be the only location in the Mediterranean Sea, or even worldwide, where aegagropilous forms of *C. prolifera* occur in abundance. Based on the high associated biodiversity, we propose the inclusion of these populations in the conservation management plans in the area.

Keywords: *Cladophora prolifera*; ball-shaped form; marine lagoon Strunjan; northern Adriatic.

Introduction

Cladophora Kützting (Cladophorales, Cladophoraceae) is one of the largest and most common globally occurring green macroalgal genera. Nearly 200 species are accepted taxonomically (Guiry & Guiry, 2021), of which the large majority are found in marine habitats and a few in freshwater (Škaloud *et al.*, 2018). These species have a relatively simple thallus architecture being composed of branched, uniseriate filaments of multinucleate cells and attached to the substratum by rhizoids or a discoid holdfast; however, unattached cushions or ball-shaped forms have also been observed. The taxonomy of *Cladophora* has been problematic owing to its morphological simplicity, parallel evolution, and phenotypic plasticity (van den Hoek, 1963; Taylor *et al.*, 2017). Molecular phylogenetic studies have indicated that the genus is not monophyletic (Bakker *et al.*, 1994; Leliaert *et al.*, 2003). In order to account for this non-monophyly, revised classifications have been proposed, notably in Cladophoraceae, Pseudocladophoraceae, and Pithophoraceae (Boedeker *et al.*, 2012, 2016), while the taxonomy of the *Cladophora* species in the Siphonocladus clade remains unresolved (Leliaert *et al.*, 2007). The reproduction in *Cladophora* may be vegetative by fragmentation of the thallus, asexual by

zoomitospores, or sexual with diplohaplontic and isomorphic life cycle (Leliaert & Boedeker, 2007). The species can also form prominent populations in marine, brackish, and freshwater habitats, where they serve important ecological functions. Species in the genus have been regarded as ecological engineers because of the structure of their thallus and as their populations help sustain a rich diversity of benthic microalgae, bacteria, and microfauna (Zulkifly *et al.*, 2013; Prazukin *et al.*, 2020). However, some *Cladophora* species may engender nuisance blooms under nutrient pollution (Zulkifly *et al.*, 2013).

The taxonomy of the *Cladophora* species has been relatively well-studied in the Mediterranean Sea. Approximately 28 marine species (including species that are currently placed in *Lychaete* J. Agardh) are known to thrive in the basin (van den Hoek, 1963; Cormaci *et al.*, 2014), of which approximately 20 have been recorded from the Adriatic Sea (Furnari, 1999; Antolic *et al.*, 2001).

Cladophora prolifera (Roth) Kützting is a perennial species that grows on the hard substrata of the lower midlittoral and upper infralittoral zone and in sheltered or slightly exposed shores. It produces dark green (blackish when dried), dense, coarse, and stiff tufts of densely branched fastigiate filaments. The branch system is acropetally organised, and the growth occurs by the division

of apical cells only; 1-3 branches are formed per cell with a small angle of ramification. The basal cells are typically elongated, and club shaped. Generally, rhizoids are formed at the basal poles of the cells of the basal and middle part of the thallus and possess characteristic annular constrictions. These rhizoids grow down along the cells below, where they entangle and form a conspicuous stipe that attaches itself to the substratum (van den Hoek & Chihara, 2000; Battelli, 2000; Leliaert & Coppejans, 2003; Leliaert & Boedeker, 2007; Sfriso, 2010; Falace *et al.*, 2013; Cormaci *et al.*, 2014). The species was originally described as *Conferva prolifera* by Roth (1797) “in mare Corsicam” and transferred to *Cladophora* by Kützing (1843). Because the type is lost, a specimen collected by Hauck at Miramare near Trieste (Italy, Adriatic Sea) has been designated as the neotype (van den Hoek, 1963). *Cladophora prolifera* is widely distributed from tropical to warm-temperate seas along the Atlantic European coasts and from Great Britain, Spain, and Portugal to northern Africa, Indian, and the Pacific Oceans (van den Hoek, 1963; Leliaert & Boedeker, 2007). It also pervades the Mediterranean coastal areas (Leliaert & Boedeker, 2007; Cormaci *et al.*, 2014). The typical attached form of *C. prolifera* is reported from the Gulf of Trieste (Falace *et al.*, 2013) and has been found in several locations in the Slovenian coastal waters: Koper Bay (Avčín *et al.*, 1973, 1974; Matjašič *et al.*, 1975; Vukovič, 1982; Battelli, 1997; Lipej *et al.*, 2004; Battelli, 2016), Strunjan bay (Avčín *et al.*, 1973, 1974; Turk & Vukovič, 1994; Lipej *et al.*, 2004), and Piran Bay (Matjašič *et al.*, 1975; Vukovič, 1980; Munda, 1993; Battelli, 1997; Lipej *et al.*, 2004).

The present paper reports the occurrence of extensive ball-shaped aggregates of *C. prolifera* in the marine lagoon of Strunjan (Gulf of Trieste, northern Adriatic). The study aims to provide general information regarding the extensive development of the mobile, free-living rolling balls of this alga observed in the lagoon in the spring of 2019 and the winter of 2021. Species identification was based on molecular and morphological data. The details pertaining to the distribution and morphology of the ball-

like form of this alga have been reported. The probable factors that resulted in the formation of the ball-shaped form of this cladophoralean species have been discussed.

Material and Methods

Study area

The lagoon of Strunjan is in the eastern part of Strunjan bay (45.528° N, 13.605° E) (Fig. 1A). It is primarily a shallow lagoon of about 10 hectares and divided into two sub-basins: a larger northern main Stjuža lagoon and a smaller south-western flowing lagoon (Fig. 1B). The flowing lagoon is a shallow environment that connects the main Stjuža lagoon with the sea by a canal approximately 5 m long and 20 m wide (Lipej *et al.*, 2019). Both the sub-basins are characterised by meadows that predominantly consist of the seagrasses *Cymodocea nodosa* (Ucria) Ascherson and *Zostera noltei* Hornemann, which are arranged in a mosaic-like pattern in the flowing lagoon (Šajna & Kaligarič, 2005; Lipej *et al.*, 2019). A total of 15 macroalgal taxa have been recorded in the area which grow attached to the substrate or unattached (Lipej *et al.*, 2019; Battelli & Gregorič, 2020). Today, the lagoon area is a significant part of the Strunjan Stjuža Nature Reserve, the primary objective of which is to preserve the biodiversity in the region.

Environmental parameters

Because of its shallow depth of approximately 0.5-1 m, thermal conditions in the lagoon of Strunjan vary seasonally: between 5° C and 10° C in the winter and between 24° C and 27° C during the summer. Owing to the substantial water exchange of the lagoon with the Strunjan bay, salinity and oxygen concentrations in the lagoon are similar to that of the bay, although the lagoon receives some freshwater inputs through small channels originat-

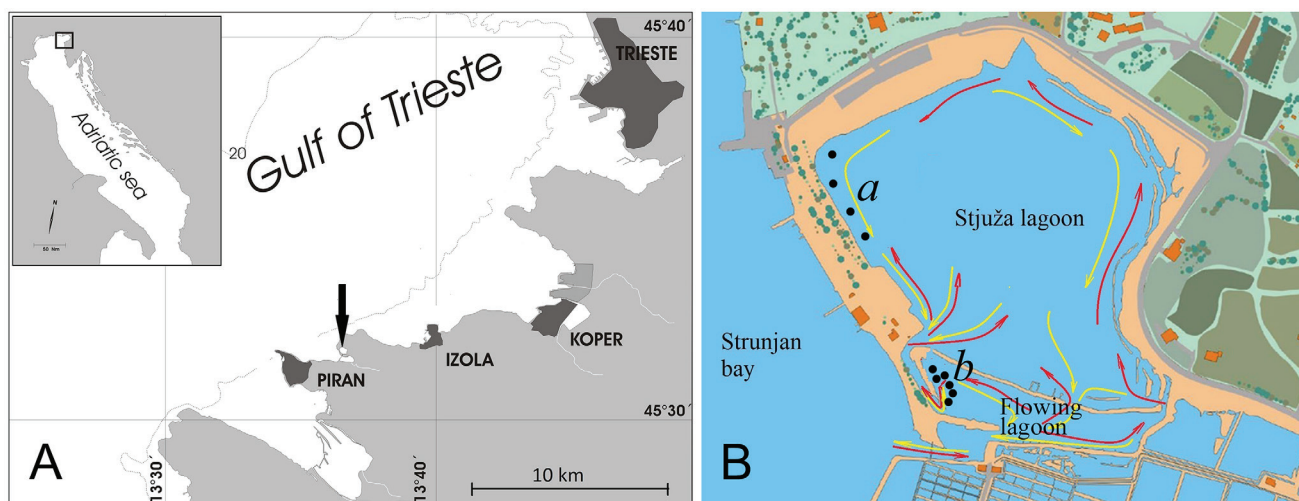


Fig. 1: A. Map of the study area. B. The distribution of ball-shaped cladophoralean species in different sites (a and b) occurring in the Stjuža and the flowing lagoon of Strunjan and the direction of the currents of the seawater during tide (after Avčín *et al.*, 1973); the yellow arrows indicate the outflow, and the red arrows indicate the inflow during the tidal change.

ing from agricultural areas (Vrišer, 2002).

The lagoon is characterised by soft sediments comprising compact-fine argillaceous silt with a slight admixture of sand and a thin (0.5-1 cm) yellowish-brown layer of flocculent organic detritus (Vrišer, 2002). The upper 15 cm of the substrate predominantly consists of brown silty clay containing snail shells and organic remains that primarily come from seagrasses and their rhizomes (Šmuc, 2020). Large accumulations of this detritus are caused by the wind and the tidal currents (Fig. 1B). The average tidal amplitude for the 1961–2020 period was 67 cm due to gravitational influences of the sun and the moon, with a high tide of 35 cm above the mean sea level and a low tide of 31 cm below the mean sea level (Slovenian Environment Agency, ARSO, www.arso.gov.si/; http://www.arso.gov.si/vode/podatki/amp/H9350_g_1.html).

Sampling procedure and data analysis

The fieldwork was undertaken when dense aggregations of the ball-shaped cladophoralean species, several centimeters in diameter (Fig. 2A, 2B), were observed in the Stjuža lagoon and the flowing lagoon in the spring of 2019. The study was conducted at site “b” of the flowing lagoon (Fig. 1B) in two periods: the spring of 2019 and the winter of 2021. This site was chosen since it exhibited a high density of ball-shaped cladophoralean species.

A field survey was conducted in the flowing lagoon to identify the habitat and determine the total coverage of the ball-shaped cladophoralean species. A small number of spherical aggregations were collected and placed in plas-

tic bags with seawater, after which they were transported to the Laboratory of the Faculty of Mathematics, Natural Sciences, and Information Technologies (FAMNIT) of Koper for further observations. The collected samples were dried, pressed, and preserved in the personal herbarium of the first author (C.B.) and the voucher specimens were deposited at the FAMNIT laboratory in Koper and the herbarium of the Meise Botanic Garden (BR, <http://www.botanicalcollections.be/>) (BR5010175907818V and BR5010175906781V).

Morphological observations and measurements were performed using an Olympus SZ61 stereo microscope with an XC50 digital camera. The diameter and length of the apical cells, main axis cells, and the basal cells (i.e., the first cell before ramification) were measured and the length/width ratio was calculated. Further, we collected samples of the attached form of *C. prolifera* from the Strunjan bay in order to compare the characteristics of the aegagropilous and the attached form of this alga. Salinity, water temperature, pH, oxygen content (O₂), and the redox potential were measured in the flowing lagoon and the Strunjan bay with a Multiparameter Waterproof Meter Hanna HI98194.

The collected algal specimens were identified to the species level whenever possible and to the genus level when diacritical features were lacking. The main studies referred to for the purpose of species identification included Maggs & Hommersand (1993), Babbini & Bressan (2003), Brodie *et al.* (2007), Sfriso (2010), and Cormaci *et al.* (2014). The nomenclature followed in this study was derived from Guiry & Guiry (2021).

Five randomly selected sampling quadrates (20 cm x

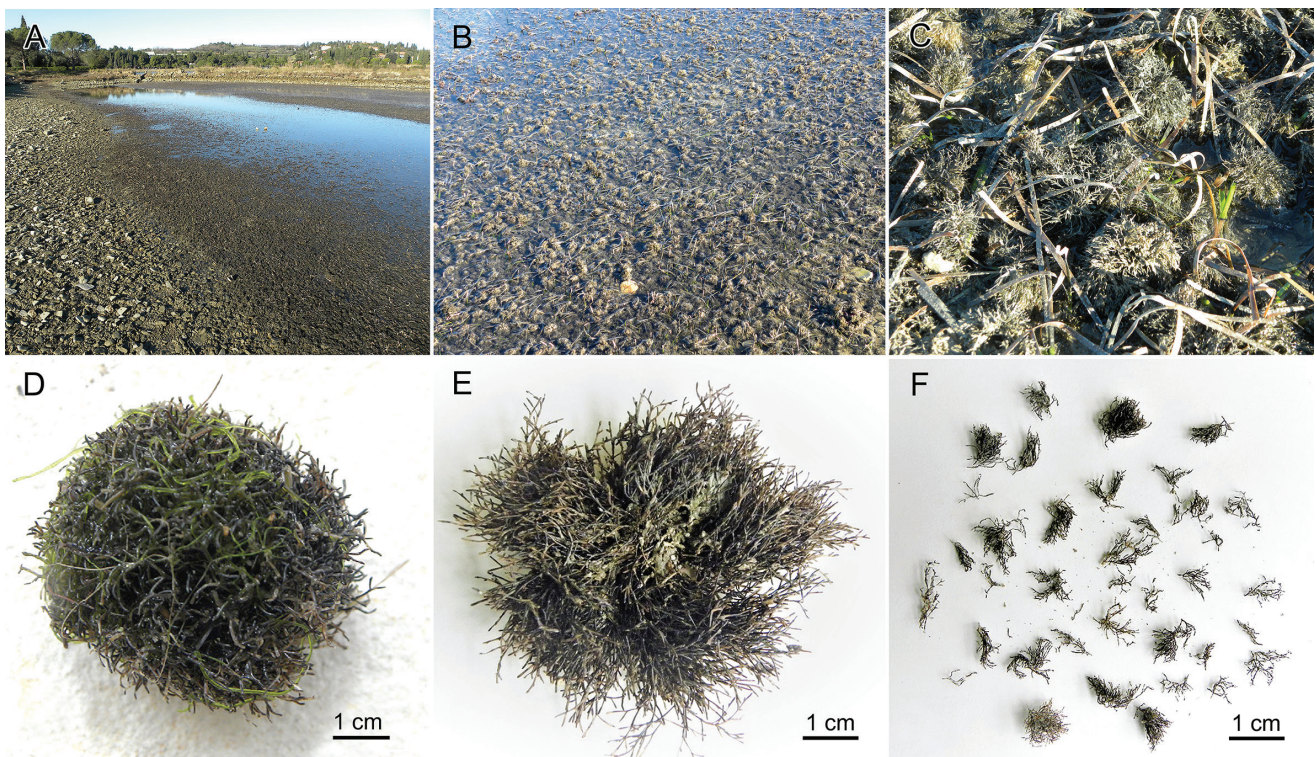


Fig. 2: A-B. Extensive aggregates of ball-shaped *Cladophora prolifera* in the flowing lagoon of Strunjan. C. Detail of the aggregates. D. The ball-shaped form of *C. prolifera*. E. A section through a *C. prolifera* ball with radially arranged branches. F. The fragments of branched filaments which comprise the balls of *C. prolifera*.

20 cm) were employed to determine the density of the cladophoralean balls at site “b” of the flowing lagoon in the spring of 2019 and in the winter of 2021.

Thirty cladophoralean balls were randomly collected from the site “b” in the spring of 2019 and the winter of 2021 to measure their diameter to the nearest 0.1 mm by utilising a caliper.

DNA-based species identification was based on partial large-subunit (LSU) rDNA sequence data. DNA from silica-dried material was extracted through a modified CTAB protocol and the first ~590 base pairs of the large subunit (LSU) rDNA were amplified by utilising the primers C'1FL and D2FL (Leliaert *et al.*, 2007). The PCR products were sent for sequencing to Macrogen (Seoul, South Korea). Sequences were submitted to the European Nucleotide Archive (ENA) under the study number PRJEB45893, and the sequence accession numbers OU246909, OU246910, OU375374, and OU375458. BLAST searches indicated *Cladophora prolifera* as a possible identification. To corroborate the DNA-based species identification, a dataset of *C. prolifera* sequences and two outgroup sequences (*Cladophora wrightiana* and *C. aokii*) were assembled, including four sequences generated in this study and publicly available sequences (www.insdc.org). Sequences were aligned by utilising MUSCLE (Edgar, 2004). A maximum likelihood (ML) and rapid bootstrap analysis was performed through RAxML v8.2.12 (Stamatakis, 2014) under the GTRCAT model on the CIPRES Science Gateway v3.3 (Miller *et al.*, 2010). Phylogenetic trees were visualised in Mega7 (Kumar *et al.*, 2016).

Results

Dense aggregates of ball-shaped cladophoralean green algae were observed among the seagrasses *Cymodocea nodosa* and *Zostera noltei* in the shallow lagoon of Strunjan in the spring of 2019 and the winter of 2021. During low tide, the aggregations stuck out partially above the water (Fig. 2A-C). Based on the molecular and morphological data (details below), we identified this green alga as *Cladophora prolifera*. The ball-shaped thalli were dark green in colour, roughly spherical, and firm in texture (Fig. 2D), composed by abundantly branched, intertwined, and radially arranged filaments (Fig. 2E, F). We were not able to demonstrate whether the ball-shaped aggregates were composed of one or multiple individual

Table 1. The density and the diameter of ball shaped *Cladophora prolifera* from the flowing lagoon (site “b”) in the spring of 2019 and the winter of 2021.

	spring 2019	winter 2021
Density (N/400 cm²)		
Min-max	24-35	32-39
Mean, SD	30.8, 5.0	35.4, 2.7
Diameter (mm)		
Min-max	18.8-38.9	19.2-48.0
Mean, SD	28.0, 4.6	29.3, 6.42

thalli. No attached forms of *C. prolifera* were found in the area. The balls also contained fragments of degraded leaves of *C. nodosa* and *Z. noltei* and were thoroughly impregnated with sediment. Some species of Rhodophyta (mainly filamentous Rhodomelaceae with *Polysiphonia*-like morphology) and Chlorophyta (mainly Cladophoraceae and *Ulva*) were found between the branches of *C. prolifera*. The mean diameter and the mean density of the ball-shaped *C. prolifera* from the study area are summarised in Table 1. The average density of the ball-shaped form of *C. prolifera* was slightly higher in the winter of 2021 compared to the spring of 2019. Moreover, larger diameters were observed in the winter of 2021.

The branching was acropetally organised and became irregular in older parts of the thallus (Fig. 3A, B). Lateral branches usually arising from the subapical cell and lower down with one or commonly two (opposite) or occasionally three to five laterals from each parent cell; cross walls at first steeply inclined to parent cell, less so in older parts. The branches are initially separated at the base by a very inclined wall (Fig. 3D, E). The apical cells were cylindrical with rounded tips (Fig. 3E). The cells of the main axes were elongated and slightly clavate (Fig. 3B, C). Basal cells were rare, clavate, and sometimes with faint annular constrictions (Fig. 3C, arrow), but without any kind of attachment system or rhizoids. Cell dimensions, including their diameter and the length/width (l/w) ratio, are summarised in Table 2.

Several attached forms of *C. prolifera* were collected in the upper infralittoral zone of the Strunjan bay during the sampling period in the winter of 2021. These attached forms were rarer than the spherical ones. The dark green

Table 2. The dimensions (in mm) of apical cells, main axial cells, and basal cells between the ball-shaped form of *Cladophora prolifera* from the flowing lagoon of Strunjan and the attached form from the Strunjan bay.

<i>Cladophora prolifera</i> ball-shaped form							
	Apical cells		Main axial cells		Basal cells		Rhizoids
	Width (w) mm	l/w	Width (w) mm	l/w	Width (w) mm	l/w	Width (w) mm
Min-max	0.2-0.3	1.8-8.3	0.2-0.3	1.2-13.9	0.2-0.3	5.5-12.0	-
Mean, SD	0.2, 0.03	4.2, 1.5	0.2, 0.0	8.6, 2.7	0.2, 0.04	8.4, 2.1	-
<i>C. prolifera</i> attached form							
Min-max	0.1-0.3	2.6-5.5	0.2-0.3	8.1-18.3	0.2-0.4	10.0-19.0	0.1-0.3
Mean, SD	0.2, 0.05	4.2, 0.98	0.3, 0.04	12.0, 3.2	1.0, 0.05	15.0, 4.1	0.2, 0.05

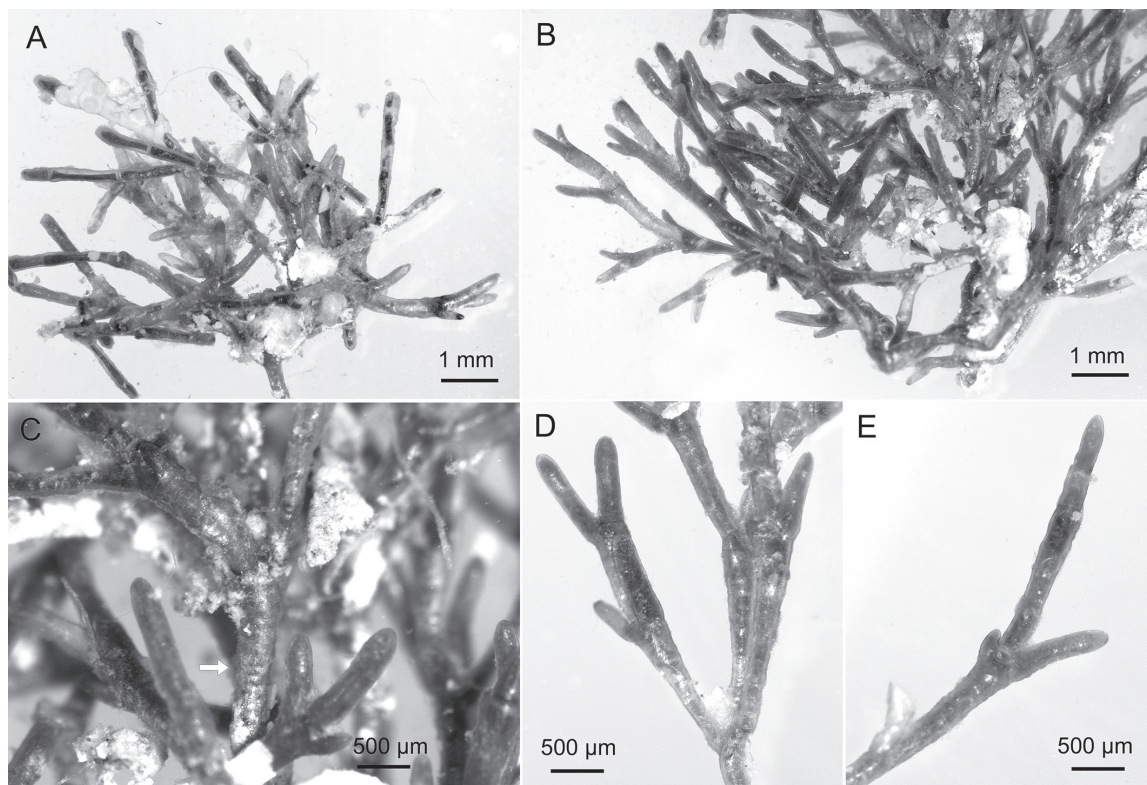


Fig. 3: *Cladophora prolifera*. A–B. Terminal, fastigate branch systems. C. Club-shaped basal cells with annular constrictions (white arrow). D–E. Detail of terminal filaments with cells producing lateral branches at acute angles.

thalli formed dense, stiff tufts of densely branched fastigate filaments, attached by rhizoids with characteristic annular constrictions at the basal poles of the cells of the basal and middle part of the thallus. These rhizoids formed a conspicuous stipe attached to the substratum. The branch system was acropetally organised; 1-3 (5) branches per cell with a small angle of ramification. The basal cells were elongated and clavate. The cell dimensions of the attached cells were comparable to the ball-shaped form (Table 2), but they differed in their l/w ratios (especially of the main axes and basal cells), which were higher in the attached form of *C. prolifera*.

In the study area, several other algal species were present in both attached and unattached forms. The most abundant macroalgal species appearing in unattached forms included the green algae *Ulva rigida* C. Agardh, *U. australis* Areschoug, *U. compressa* Linnaeus, *U. intestinalis* Linnaeus, *U. flexuosa* Wulfen, *U. kylinii* Bliding, *Chaetomorpha linum* (O.F. Müller) Kützing, *Cladophora lehmanniana* (Lindenberg) Kützing, and *C. liniformis* Kützing. The red algae comprised of *Chondria capillaris* (Hudson) M. Wynne, *Polysiphonia scopulorum* Harvey, *Polysiphonia* sp., *Polysiphonia spinosa* (C. Agardh) J. Agardh, *Ceramium* spp. The brown algae constituted *Cystoseira aurantia* Kützing and *C. foeniculacea* f. *tenuiramosa* (Ercegović) A. Gómez Garreta, M.C. Barceló, M.A. Ribera & J. Rull Lluh. *Cystoseira foeniculacea* f. *tenuiramosa* was present and attached to small pebbles and epiphytic on the ball-shaped red alga *Rytidhlaea tinctoria* (Clemente) C. Agardh.

The most abundant epiphytes on the *C. prolifera* balls were the crustose coralline red algae *Lithophyllum cystoseirae* (Hauck) Heydrich and *Titanoderma pustulatum*

(Lamouroux) Näegeli which covered most of the branches, while *Ceramium* spp. were rare.

Several small invertebrate taxa were found only on the balls, including the gastropods *Bittium reticulatum* (da Costa) and *Ecrobia ventrosa* (Montagu), various nudibranchs, the bristle star *Amphipholis squamata* (Delle Chiaje), the asteroidean *Asterina gibbosa* (Pennant), diverse polychaetes, and amphipods such as *Gammarus inaequicauda* (Stock) and *G. insensibilis* (Stock).

The LSU rDNA sequences of the spherical *Cladophora* thalli were identical to the sequences of *Cladophora prolifera* from various localities worldwide, including the Mediterranean Sea (Corsica), thus substantiating the identification of the species as *Cladophora prolifera*. The NJ tree is illustrated in Figure 4B, along with a schematic overview phylogeny of the Cladophorales (Fig. 4A) illustrating non-monophyly of the genus *Cladophora* with the bona fide *Cladophora* species belonging to the Cladophoraceae, while other *Cladophora* species (including *C. coelothrix* and *C. prolifera*) belong to the *Siphonocladus* clade.

Discussion

The unattached macroalgae, growing in more or less spherical forms, are described by the term aegagropilous, which was first used by Linnaeus (1763) to describe a ball-shaped green alga species from the Baltic Sea: *Conferva aegagropila* Linnaeus (a synonym of *Aegagropila linnaei* Kützing). The term was later utilised in a broader sense to describe loose masses of algae rolling up into balls due to the influence of the waves and the

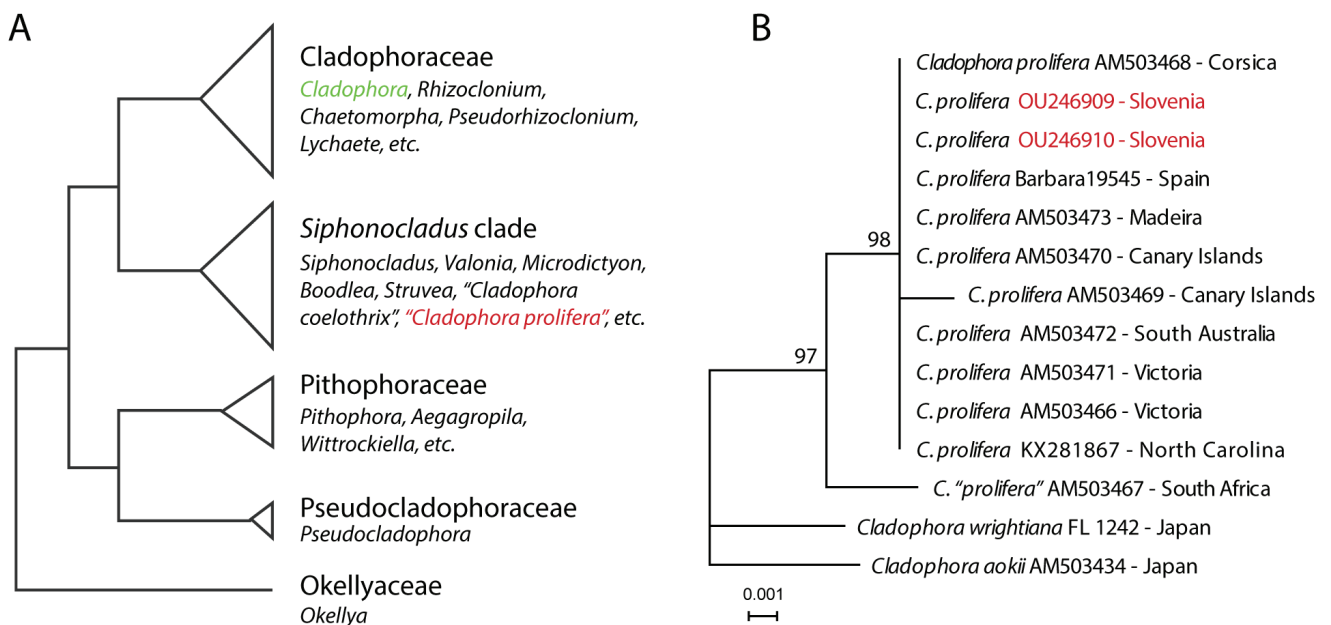


Fig. 4: Phylogenetic position of the ball forming *Cladophora prolifera* from the lagoon of Strunjan. A. Schematic phylogeny of the Cladophorales based on Leliaert *et al.* (2003, 2007) and Boedeker *et al.* (2012, 2016), illustrating non-monophyly of *Cladophora*. B. ML phylogeny based on partial large-subunit (LSU) rDNA sequence data, showing the position of the ball-forming *C. prolifera* from Slovenia.

currents. Taxonomically, the term was employed to group ball-shaped cladophoralean species in a separate genus (*Aegagropila*) or section of *Cladophora*. Kützing (1843) classified cushion- and ball-shaped filamentous green algal species in the genus *Aegagropila*, while categorising the rest under *Cladophora*. In later classifications, *Aegagropila* has been regarded as a section of *Cladophora* (van den Hoek, 1963; van den Hoek & Chihara, 2000), but molecular phylogenetic data substantiated the earlier view of a separate genus (Boedeker *et al.*, 2012). One of the best-known examples of spherical macroalgae are “lake-balls” of the species *Aegagropila linnaei* (Kurogi, 1980), but aegagropilous forms are also produced by several marine species of *Cladophora*, as well as by at least 54 other seaweeds, including 25 red, 18 green, and 11 brown algae (Norton & Mathieson, 1983; Mathieson & Dawes, 2002).

Spherical forms of *Cladophora* in Slovenian coastal waters were reported for the first time in the Strunjan bay by Avčín *et al.* (1973) under the name of *Cladophora echinus* (Biasoletto) Kützing (now *Lychaete echinus* (Biasoletto) M.J. Wynne). The aegagropilous forms were later reported from the Stjuža sea lagoon under the same name (Battelli, 1997; Battelli & Gregorič, 2020). In the present study, we have identified these algae as *C. prolifera* based on the DNA data obtained. We can therefore assume that the aegagropilous form of *C. prolifera* was already present in the lagoon in 1973 and 1997.

While the attached form of *C. prolifera* is widely distributed from the tropical to the warm-temperate seas, including the Mediterranean Sea (van den Hoek, 1963; Leliaert & Boedeker, 2007; Cormaci *et al.*, 2014), to our knowledge, there is only a single report on the occurrence of aegagropilous form of this species from a lagoon in Bermuda (Bach & Josselyn, 1978).

Plustophytic populations, rich in ball-shaped forms typical of lagoon environments, are common in the Mediterranean Sea. They include the green macroalgae *Valonia aegagropila* C. Agardh, *Lychaete echinus*, and *Chaetomorpha linum* and the red macroalgae *Alsidium corallinum* C. Agardh, *Rytiphlaea tinctoria*, *Spyridia filamentosa* (Wulfen) Harvey, and *Gracilaria bursa-pastoris* (S.G. Gmelin) P.C. Silva (Calvo *et al.*, 1980; Orestano & Calvo, 1985; Cecere *et al.*, 1992; Petrocelli *et al.*, 2009).

Two main hypotheses have been proposed for the formation of the aegagropilous cladophoralean algae. The first hypothesis considers that aegagropilous forms result from the dynamic effect of wave motion with a uniform water movement allowing the radial growth of the thallus by making it roll on the bottom, thus continuously varying its light exposure (Smith, 1950; Fritsch, 1965). The second hypothesis supports the active developmental role of the alga (Austin, 1960; van den Hoek, 1963). Boedeker & Immers (2009) argued that the formation of the ball-shaped macroalgal forms arises from a combination of mechanical processes and species-specific features that favour entanglement, such as the stiff texture and growth pattern resulting from the abrasion of apical cells as they roll over the sediment. During the course of the present study, we observed the absence of obvious regeneration phenomena due to abrasion, which indicates that the spherical structure does not result from abrasion over the bottom, but rather, is a consequence of wave-induced rolling on sandy sediments and morphological features that contribute to filament entanglement and radial growth as a result of light exposure (Kurogi, 1980; Niiyama, 1989).

Petrocelli *et al.* (2009) suggested that one of the causes for the formation of the unattached forms of the red macroalgae, *Alsidium corallinum*, *Spyridia filamentosa*, and *Gracilaria bursa-pastoris* from the lake of Varano,

Adriatic Sea, is the scarcity of hard substrates. The authors also noted that the unattached thalli differ morphologically from the conspecific attached thalli, particularly in the lack of a holdfast and the radially arranged branches. These morphological features are consistent with our findings in the spherical form of *C. prolifera*.

Based on the information available from other parts of the Mediterranean Sea, we argue that a number of environmental conditions characteristic of the Stjuža and the flowing lagoon favour the formation of the spherical form of *C. prolifera*. These include shallow water with an average depth of about 0.5–1 m, allowing continuous exposure to sunlight and thus, radial growth of algal thalli. The surface and bottom water currents generated by winds blowing mainly from the north-east (burja) and the south-east (jugo); a relatively large tidal range of about 67 cm; and a soft sediment bottom, which is unfavourable for the development of attached macroalgae.

Aggregates of aegagropilous *C. prolifera* occurred mainly in the flowing lagoon (they were rare in Stjuža lagoon). This shallow environment represents a transitional habitat from the sea to the Stjuža lagoon. Based on the available information (Avčin *et al.*, 1973; Vrišer, 2002; Lipej *et al.*, 2019), salinity, thermal conditions, pH, oxygen content, and the redox potential in the Stjuža and the flowing lagoon are associated with large water exchange with the Strunjan bay and thus, are generally similar to those of the Strunjan bay (Table 3). This is especially true for the flowing lagoon, which is directly connected to the Strunjan bay by a large channel. This sea connection enhances water circulation and probably promotes colonisation and the spread of aggregations of the ball-shaped form of *C. prolifera*. In addition, since this alga is unattached, water circulation patterns in the Strunjan lagoon undoubtedly influence its distribution toward this portion of the Stjuža lagoon. The sea connection also ensures better circulation of water masses in the Strunjan lagoon, consequently reducing organic pollutants.

For these reasons, we do not consider the occurrence of the aegagropilous masses of *C. prolifera* as a disruptive algal bloom (green tide) proliferating due to anthropogenic factors (e.g., increased nutrient levels). In our opinion, these populations do not pose a threat to the existing ecosystems that mainly consist of the seagrass meadows of *C. nodosa* and *Z. noltei*, where these aggregations occur.

We hope that the present study will stimulate further research on the biophysical features and the ecological importance of these aegagropilous populations.

Conclusions

In conclusion, we argue that the formation of the aegagropilous *Cladophora prolifera* in the marine lagoon of Strunjan is a consequence of a) the confluence of the tidal currents and water current caused by the wind, which prevents the occurrence of the stagnation phenomena and determines favourable hydrodynamic conditions for the development of the spherical forms, rolling on the bottom and thus, continuously varying their exposure to light and b) the morphological characteristics that mainly comprise of the densely branched filaments, which favour their interlacing and enhances their compactness.

According to the available information, the Strunjan marine lagoon is apparently the only location in the northern Adriatic where aegagropilous forms of *C. prolifera* occur. Thus, our results contribute by enhancing the understanding of unattached, ball-shaped forms of algal populations in this area. Based on the substantial biodiversity present in the surroundings, we propose that the aegagropilous form of *C. prolifera* from the marine lagoon of Strunjan should be considered for incorporation in the conservation management plans.

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Table 3. Values of salinity, temperature, pH, oxygen, and redox potential from the sampling site “b” (flowing lagoon) and Strunjan bay.

Location	salinity	T (°C)	pH	O ₂ (ppm)	Redox (mV)
flowing lagoon	34.00	19.64	7.87	10.17	7.7
Strunjan Bay	34.46	16.26	8.25	11.17	36.9

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