



Living Inside a Jellyfish: The Symbiosis Case Study of Host-Specialized Dinoflagellates, “Zooxanthellae”, and the Scyphozoan *Cotylorhiza tuberculata*

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The photosymbiosis with host-specific dinoflagellates is a widespread relationship in marine organisms. Despite the evidenced biodiversity of this kind of mutualism, most research focuses on the study of scleractinian corals, and there is a lack of knowledge about other symbiotic cnidarians such as jellyfishes. The Mediterranean jellyfish *Cotylorhiza tuberculata* (Rhizostomae, Scyphozoa) harbors an endosymbiotic dinoflagellate of the family Symbiodiniaceae. In this study, we examine the algae distribution within the host body as well as, the pigment content and cell density of the symbiont. Furthermore, the size, morphology and fluorescence of cultured symbionts were studied under light microscopy, Imaging Flow Cytometry (IFC), and Scanning Electron Microscopy (SEM). The C:N composition and optical properties of the medusa tissue were measured to evaluate their role in the symbiosis. The medusae body was divided into two different sections to investigate the distribution of symbionts *in hospite*: oral arms (OA) and umbrella (UM). C:N composition of *C. tuberculata* was and symbiont density was significantly higher in the OA section. Mean chlorophyll *a* concentration of the algae was $1.33 (\pm 0.83) \text{ pg Chl } a \text{ cell}^{-1}$. The study of the pigment composition by HPLC (High Performance Liquid Chromatography), revealed the presence of 13 different pigments, being the most representative chlorophyll *a*, chlorophyll *c*₂, and peridinin typical pigments of Symbiodiniaceae. Cell diameter of algae freshly isolated from the host was $8.71 \pm 0.97 \text{ }\mu\text{m}$ and cell growth rate was $0.52 (\pm 0.09) 10^6 \text{ cell ml}^{-1} \text{ d}^{-1}$. The presence of vegetative coccoid cells, doublet and motile mastigotes were revealed within the Symbiodiniaceae cultures. A calcifying matrix typical of Symbiodiniaceae and formed in partner with bacteria, was also observed most cultures. The umbrella tissue of the medusa absorbed at ultraviolet radiation (UVR) region, suggesting that medusae tissue protects photosymbionts from the negative effect of the high energetic UVR and

attenuates the light intensity reaching algae inside the host. The presence of a dense Symbiodiniaceae population and the protection to UVR and elevated environmental irradiance provided by medusae tissue, maintain symbionts in optimal light conditions for photosynthesis and may be a reason added to explain the population success of *Cotylorhiza tuberculata*.

Keywords: Symbiodiniaceae, Chl a, HPLC, microscope (SEM), absorbance, photosymbiosis

INTRODUCTION

The photosymbiosis with host-specialized dinoflagellates is a widespread relationship in marine organisms. This mutualistic association between animal hosts (heterotrophic) and endosymbiotic dinoflagellates (autotrophic) is found in cnidarians, sponges, molluscs, and even in protists (Venn et al., 2008; Decelle et al., 2015). Despite the large diversity in photosymbiosis, most work focus on the study of scleractinian corals, the basis of coral reefs and one of the most important and threatened ecosystems in the world (Done et al., 1996; de Groot et al., 2012; Kennedy et al., 2013). In contrast, other symbiotic cnidarians, such as jellyfishes, have been poorly examined (Djeghri et al., 2019). Nevertheless, due to the rise of jellyfish population in some environments (Arai, 2001; Pérez-Ruzafa et al., 2002; Purcell, 2005), there is a growing interest in studying these symbiotic organisms belonging to the cnidarian group and characterize their role in the ecosystem (Djeghri et al., 2019).

The Mediterranean jellyfish *Cotylorhiza tuberculata* is one of those symbiotic scyphozoans whose abundance has increased over the past decades. High abundances of this species have been observed in enclosed marine areas such as the Vlyho Bay in Greece (Kikinger, 1992) and the Mar Menor coastal Lagoon in Spain, where annual blooms have been observed since the 1990s (Pérez-Ruzafa et al., 2002). Like most scyphozoans, *C. tuberculata* has a complex bipartite life cycle with alternation of a pelagic sexual stage (medusa) and a benthic asexual stage (polyp). In this species, the endosymbiotic association occurs early in the jellyfish life cycle, particularly during the polyp stage (Astorga et al., 2012). In addition, the presence of host-specialized symbionts seems to play an important role in the phase transition from sessile polyp to free-living medusae, known as strobilation (Kikinger, 1992; Prieto et al., 2010; Astorga et al., 2012). The symbiotic dinoflagellates are hosted in the jellyfish endodermal tissue (Kikinger, 1992). As in most symbiotic jellyfishes, *C. tuberculata* polyps acquire their symbionts from the environment by horizontal transmission (Kikinger, 1992; Astorga et al., 2012). Then symbionts are incorporated into their endodermal cells (*via* lysosomes) and many of those cells containing symbionts become mesogleal amoebocytes filled with algae (Colley and Trench, 1985). Once a polyp strobilates into an ephyra, the algal cell population inside the amoebocytes multiply and accumulate close to the endoderm (Lajeunesse et al., 2009). In adults of *C. tuberculata*, symbiotic dinoflagellates are distributed along the gastrovascular system indicating nutritional importance (Kikinger, 1992).

Host-specialized dinoflagellates associated with jellyfish species belong to the family Symbiodiniaceae

(Lajeunesse et al., 2018) which comprises numerous genera and species with large differences in diversity, ecology, and geographic distribution. Symbiosis between medusozoans and Symbiodiniaceae originated independently at least seven times, with five of these originations involving groups that contain pelagic taxa with three of them having a medusae phase (cubozoa, hydrozoa, and scyphozoan; reviewed in Djeghri et al., 2019). Within the class Scyphozoa, the orders Coronatae (with the family Linuchidae and some *Nausithoe* species) and Rhizostomeae (in the suborder Kolpophorae) are known to host symbiotic dinoflagellates species, with 20–25% of scyphozoan species being photosymbiotic. However, the majority of the studies about symbiotic jellyfish has concentrated on species of the genus *Cassiopea* (Fitt and Trench, 1983; Banaszak and Trench, 1995; Verde and McCloskey, 1998; Thornhill et al., 2006; Mortillaro et al., 2009; Lampert et al., 2012; Mellas et al., 2014; Leblond et al., 2015). In fact, the “upside-down” jellyfish *Cassiopea* spp., has been used as a model organism of cnidarian-dinoflagellate symbiosis (Ohdera et al., 2018) despite having an unusual benthic lifestyle that differs from the rest of zooxanthellate jellyfish, with a pelagic lifestyle. Therefore, there is still a significant lack of knowledge on zooxanthellate jellyfish ecology, and basic information on the role of symbiosis for most species is still scarce.

The symbiosis benefits both, the host and the symbiont. Algae produce organic compounds photosynthetically that are passed to the host medusa. In return, the host provides the symbiont with nitrogen and phosphorus (Ohtsuka et al., 2009). Zooxanthellate jellyfishes in their medusa stage obtain the major part of their nutritional energy from the photosynthesis carried out by the symbiont (Djeghri et al., 2019). Many studies conducted in symbiotic scyphozoans support the idea that photosynthetic rates are often equal or superior to respiration rates (Kremer et al., 1990; Kikinger, 1992; McCloskey et al., 1994; Verde and McCloskey, 1998).

The important role of the symbionts in jellyfish nutrition suggests that the host may present some behavioral and morphological adaptations to maintain their photosynthetic partners under the best light conditions (Djeghri et al., 2019). It is already known that zooxanthellate jellyfish perform complex horizontal and vertical migrations (Kikinger, 1992; Dawson and Hamner, 2003) or circadian regulated tissue contractions to guarantee illumination and optimize photosynthesis. Furthermore, some evidence indicates that jellyfish have developed morphological adaptations to protect the photosynthetic apparatus of the symbiont against the potentially damaging UV radiation (Blanquet and Phelan, 1987) although recent molecular studies do not support this hypothesis

(Lampert et al., 2012). Not only the jellyfish tissue protects Symbiodiniaceae, but also the dinoflagellate's pigments have an important role in the protection of the photosynthetic apparatus (Jeffrey and Haxo, 1968; Jeffrey et al., 2011).

The characteristics of the symbiont population of the medusa *C. tuberculata* have not often been studied. Symbionts have been genetically identified as *Symbiodinium* A1 (Lajeunesse et al., 2009) and *Symbiodinium* Temperate A (Visram et al., 2006). However, specimens isolated from the Mar Menor used in this work, seem to host two genetically distinct Symbiodiniaceae genus: *Breviolum* and *Philozoon* (Raspor Dall'Olio, 2016; LaJeunesse et al., 2021).

Here, we examined the distribution and density of Symbiodiniaceae in adult specimens of *C. tuberculata*. Pigment content, size and morphology, growth and carbon production of the symbiotic population were also characterized after isolation from host and maintenance in culture. The medusa tissue optical properties were also studied to evaluate the symbiont light conditions inside the host and the role played by the jellyfish tissue in the symbiosis.

MATERIALS AND METHODS

Sampling

C. tuberculata adult specimens were collected at the Mar Menor coastal lagoon (37°44'N 0°47'W), on the southeastern shore of the Iberian Peninsula (Spain) (Figure 1). Samples were captured in October 2010, when the pelagic medusa stage of *C. tuberculata* thrives in the water column (Prieto et al., 2010). A total of 47 adult medusae were trapped using a net and transferred to large coolers filled with surrounding seawater and aeration. Samples were subsequently transported to the laboratory to be immediately processed.

Jellyfish Biomass and Elemental Composition

The diameter of the umbrella and wet mass (WM) of each captured individual ($N = 47$) was measured by carefully extending them and daubing-off excess water. In total, 43 medusae were used to characterize algae distribution within the jellyfish body, pigment content, and C:N composition. The remaining 4 specimens were used for analyses of the tissue absorbance.

Jellyfish specimens ($N = 43$) were divided into two distinct sections (Figure 2): Umbrella (UM, including umbrella and gastric tissue) and oral arms (OA, which included the moutharm canal and appendages, and the brood-carrying filaments). The total volume of each medusa section was determined by separately homogenizing the jellyfish tissues using a manual mixer. From the known total homogenate volume, 12 samples of 10 ml were taken: six from OA section and six from UM.

To determine the jellyfish biomass and biochemical composition, triplicate samples of 10 ml of each section were used. Samples were transferred to Petri dishes for wet mass (WM) measurements and oven dried at 100°C to a constant dry mass (DM). Then, C:N composition was further determined

using a TOC-V_{CPH/CNP} analyzer (Shimadzu) and expressed as percentage of dry mass.

Host-Specialized Dinoflagellates Isolation

Symbiodiniaceae were isolated from the homogenized jellyfish samples with two purposes: (1) analysis of the symbiont distribution and pigments and, (2) assessment of their growth and maintenance in culture. Freshly isolated algae were obtained by three centrifugations at maximum speed (5,000 rpm). After each centrifugation, the jellyfish tissue debris and supernatant were discarded, and the algal pellet was resuspended in sterilized and filtered seawater. Finally, the obtained algal suspension was filtered through a 55 µm mesh, recentrifuged once and resuspended in a final volume of 10 ml filtered seawater to obtain the algal population. The same process was followed for the isolation of symbionts to set up algal cells cultures, although in this case, the pellet was resuspended in sterilized filtered seawater containing *f/2* (Guillard and Ryther, 1962) medium. Cultures maintenance and analysis are described in section "Cultured Symbiodiniaceae."

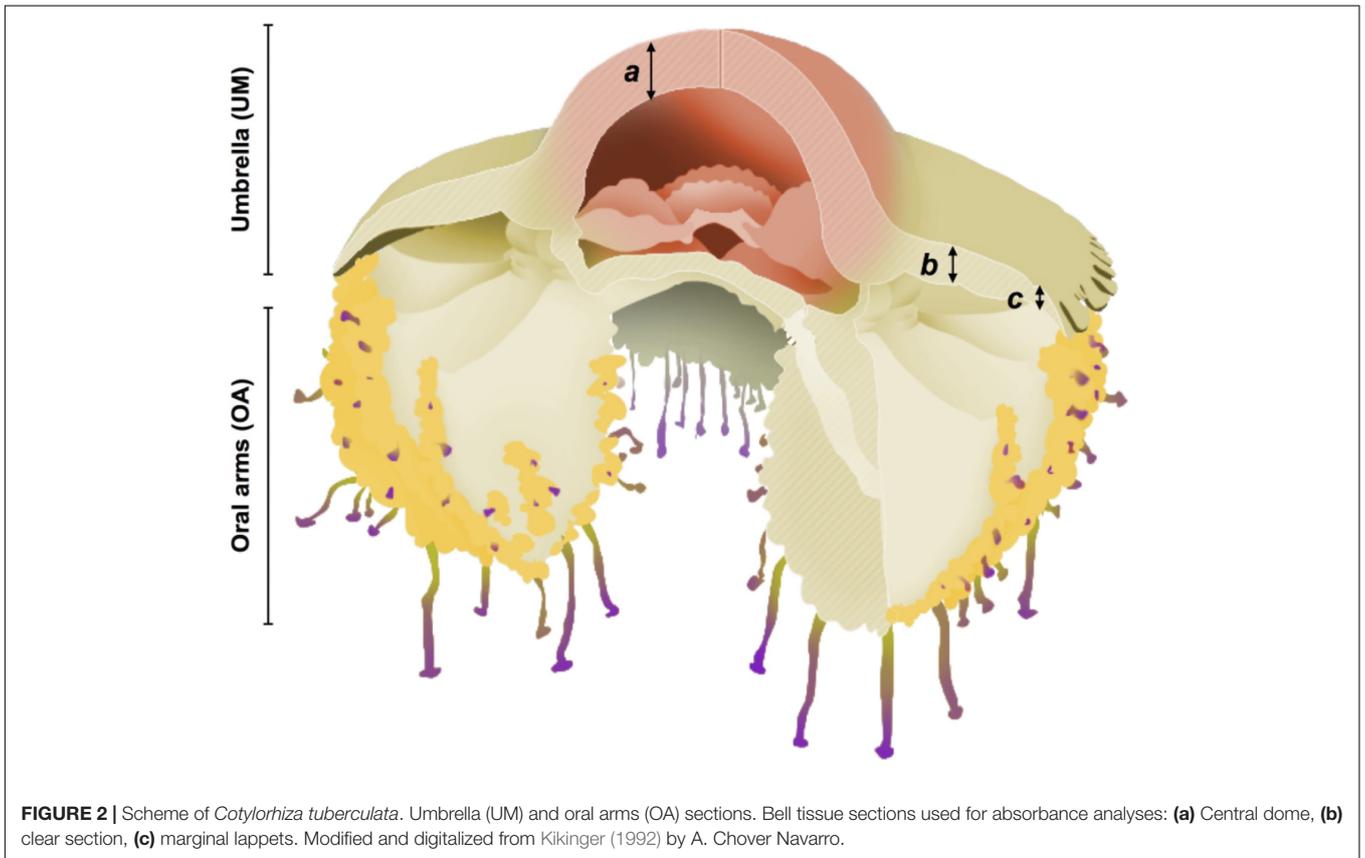
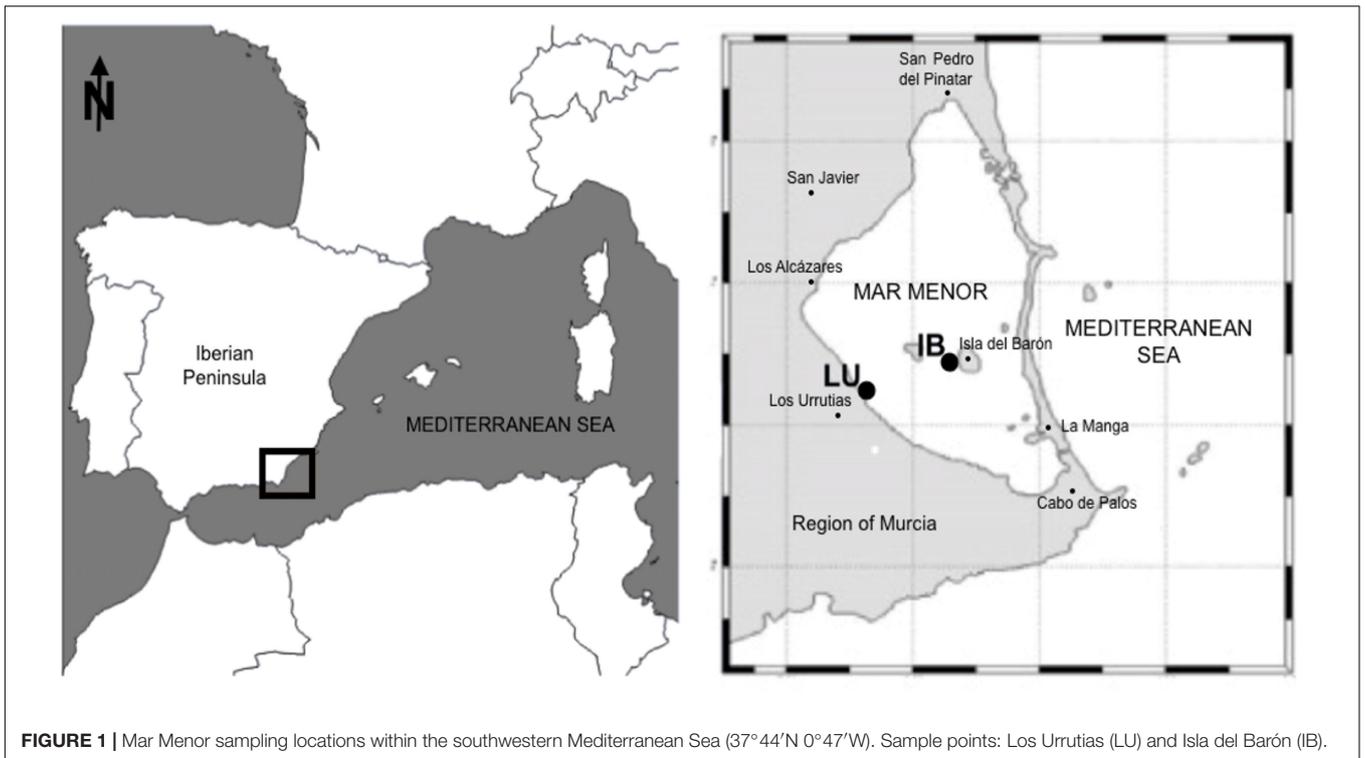
Symbiont Population and Pigment Content

To characterize the Symbiodiniaceae population, freshly isolated microalgae suspension was used for cell counts and size measurements in the different jellyfish sections. Then, pigment content was analyzed using two different techniques: (1) Fluorometry to measure total Chl *a* and phaeopigments, and (2) High Performance Liquid Chromatography (HPLC), to quantify and identify pigments content.

Cell counts and size measurements were carried out under optical microscopy. Triplicate samples of 1.5 ml of homogenate volume per medusa and section were used for counts. From these samples, the diameter of 10 randomly chosen cells was measured. After manipulation, algae were fixed in 4% formaldehyde to avoid cell shape damage (McAuley and Smith, 1995).

Chlorophyll *a* and phaeopigments quantification were measured fluorometrically using a Turner Designs 10-AU Model fluorometer according to the Joint Global Ocean Flux Study (JGOFS) protocol (Knap et al., 1996). Triplicate samples of 1.5 ml of algal suspension per medusa and section were filtered through a GF/F Whatman filter. Then, samples were sonicated into 90% acetone to extract pigments and maintained overnight at dark and 4°C until analyses. The fluorometer was calibrated using a pure Chl *a* standard from the cyanobacterium *Anacystis nubilans* (Sigma Chemical Company).

The separation and quantification of photosynthetic pigments were determined using the reverse-phase HPLC method (Mantoura and Llewellyn, 1983; Barlow et al., 1993). Triplicate samples of 10 ml algal suspension from 11 *C. tuberculata* individuals were analyzed. Samples were filtered (47 mm GF/F Whatman filters), placed in 4 ml of acetone 100% and sonicated for pigment extraction, and finally centrifuged to remove cellular debris and particles. The pigments content was identified by



comparing the obtained HPLC profiles and their retention times with those of pure standards (DHI Danmark).

Cultured Symbiodiniaceae

Freshly isolated Symbiodiniaceae were maintained in culture with *f/2* as growth medium (Guillard and Ryther, 1962) prepared with sterilized and filtered (0.2 μm) seawater. Cultures were kept in a culturing chamber at 20°C and pH 8.0 under 60 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ and 16 light:8 dark photoperiod. As soon as growth was observed, strains were frequently transferred to a new sterilized culture medium to assure relatively clean algae.

The study of the cultured symbiont population and cell morphology was conducted using different techniques and tools. Light microscopy, differential interference contrast microscopy (DIC) and imaging flow cytometry (IFC), were used to study Symbiodiniaceae *in vivo* whereas Scanning Electron Microscopy (SEM) was applied to examine the symbiont morphology.

Symbiodiniaceae population was first observed under light microscopy after settling by Utermöhl technique using inverted microscopy at 10 \times , 20 \times , and 40 \times magnification. Then, symbiont samples were analyzed by Image Flow Cytometry (IFC) using an Image Stream X Mark II (Amnis, Seattle, United States) equipped with 20 \times , 40 \times , and 60 \times objectives and two lasers emitting at 488 and 652 nm. Samples were run at low speed acquiring 2,000 cells per sample.

Additionally, Symbiodiniaceae samples for SEM were previously fixed in 2% OsO₄ for either 10–15 min and placed on poly-L-lysine coated coverslips or 25 mm black polycarbonate filters of 0.2 μm pore size (GE Water and Process Technologies). After washing in dH₂O for 30 min, samples were dehydrated in an EtOH series: 30, 50, 70, 96, and 99.9% (10 min in each change) and two changes of 100% EtOH (30 min in each change), a final change was conducted in acetone 100%. Samples were critical point dried. The filters or coverslips were mounted on stubs, coated with palladium-platinum and finally examined in a Nova NanoSEM 450 (FEI, Field Electron and Ion Company, OR, United States) scanning electron microscope.

Photosynthesis vs. Irradiance

Photosynthesis vs. irradiance (P-I) experiments were carried out using a photosynthetron Lewis and Smith (1983). A volume of 1,000 ml of Symbiodiniaceae culture was spiked with 100 μl ¹⁴C-bicarbonate and split into 50 clean glass scintillation vials (20 ml). Then, vials were incubated for 2 h and illuminated with light intensities ranging from a few to $\sim 1,200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Two scintillation vials were maintained in the dark to quantify respiration. Illumination was provided by a tungsten-halogen lamp with a blue-CuSO₄ filter mounted between the lamp and the samples. Filters were used to attenuate the light intensity and achieve the desired range of light levels. Light intensity in the photosynthetron sample chambers was measured with a Biospherical PSP-100 sensor. Incubation temperature was 19.5°C. After incubation, samples were filtered through Whatman GF/F glass fiber filters (25 mm) under vacuum pressures < 100 mmHg. Then, labeled organic carbon retained in the filters was measured to estimate the total primary production (PP) (Moran and Hodson, 1990). After 2 h formalin was added

to stop the incubation. To eliminate by vaporization all unincorporated ¹⁴C, sample filters were exposed to 750 μl fuming HCl for 12 h prior to the addition of 10 ml of scintillation cocktail (Ultima Gold, Packard). The ¹⁴C radioactivity was determined on a liquid scintillation counter (Beckman LS 5000CE). Disintegrations min^{-1} (dpm; 1 dpm = 0.0167 Bq) were calculated by the external standard method. Before calculations, black vials values were subtracted from the light vials values for correction of non-photosynthetic ¹⁴C fixation. Since no photoinhibition was observed, photosynthetic parameters of the P-I curve were determined by non-linear least-squares regression, fitting the data to the model of Webb et al. (1974):

$$P^B = P_m^b \left[1 - e^{(-\alpha I/P_m^b)} \right]$$

where P^B (mgC mg Chl⁻¹ h⁻¹) is the chlorophyll *a*-normalized photosynthetic rate, P_m^b (mgC mg Chl⁻¹ h⁻¹) correspond to the maximum chlorophyll *a*-normalized photosynthetic rate and α^b [mg C mg Chl⁻¹ h⁻¹ ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$)⁻¹] represents the chlorophyll *a*-normalized initial slope of the P-I curve.

Optical Properties of the Host Jellyfish Tissue

Four adult specimens of *C. tuberculata* were maintained alive and destined to measure the tissue optical properties of the intact association. The jellyfish umbrella was divided into three different sections according to coloration (**Figure 2**): (a) central dome (yellow-brown), (b) clear section (thick and white), (c) marginal lappets (thin and white).

Depending on the medusa size, two or three rectangular pieces of each section were cut from living specimens with a scalpel. Each umbrella section was individually stuck to the lateral side of a quartz cuvette. Then, samples were exposed to the different wavelengths of the light spectrum inside a UV-VIS Perkin Elmer Lambda-5 spectrophotometer (190–2,500 nm). Absorption spectra data were collected with a data collection system and corrected to exclude residual scattering by subtracting the apparent absorbance at 750 nm. Sections were oriented in such a way that spectrophotometer beam of light passed through the jellyfish umbrella tissue layers as in nature: from exumbrella epidermis (ectoderm) to mesoglea and coronal muscle and then to subumbrella epidermis. Considering that the tissue section is scattering light as well as absorbing it, we used a prisma to make a true measurement of absorbance.

To estimate light conditions inside the jellyfish the shade provided by the jellyfish tissue was measured as well as, the light intensity reaching Symbiodiniaceae in each specific part of the umbrella. Light attenuation provided by each umbrella tissue section (*a*, *b*, and *c*, see **Figure 2**) was measured in 4 living specimens of *C. tuberculata*. Firstly, surface irradiance in a laboratory aquarium and irradiance under each umbrella tissue on the surface was measured using a QSL-Radiometer (Biospherical instruments Inc.). Finally, the umbrella thickness was measured in each section to estimate the% of light attenuation provided by each tissue as a function of the tissue thickness.

The irradiance reaching the jellyfish tissue at the surface and at 1 m depth was calculated by the following equation: $I_z = I_0 e^{-kz}$, where I_z is the light intensity for a given depth (z), I_0 the light intensity at the surface and k the light extinction coefficient. The surface irradiance measured during the sampling efforts carried out in the present work was approximately $4,000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, assuming a k of 0.4 as a mean light extinction coefficient in the Mar Menor during summer-autumn (Terrados and Ros, 1991; Lloret et al., 2008), the light intensity reaching the medusa at 1 m depth in the lagoon would be $2,681 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

Statistical Analyses

Prior to analysis, all data sets were tested for compliance with the statistical test assumptions, normality, and homoscedasticity. If assumptions were violated, the data were retested, or a non-parametric test was utilized. The software package R (R Foundation for Statistical Computing) was utilized to conduct statistical and graphical analysis.

RESULTS

Host Biometrics and Elemental Composition

The diameter and wet mass (WM) (mean \pm SD) of *C. tuberculata* specimens collected from the Mar Menor ranged from 7.0 to 33.5 cm (23.9 ± 6.3) and 39 to 2,780 g ($1,332 \pm 676$), respectively. C and N content of the intact association was expressed as percentage of dry mass. C percentage in the tissues ranged from 1.9 to 10.5% (7.3 ± 1.7) in OA section and from 0.9 to 14.7% (5.3 ± 2.5) in UM section whereas N percentage represented 0.4–2.5% (1.7 ± 0.5) in the OA and from 0.4 to 3.5% (1.4 ± 0.6) in UM. The C:N ratio was significantly higher in OA (4.4 ± 0.30) than in the UM (3.8 ± 0.26) section (Mann-Whitney U -test, $U = 1,636$, $N = 43$, $P < 0.01$).

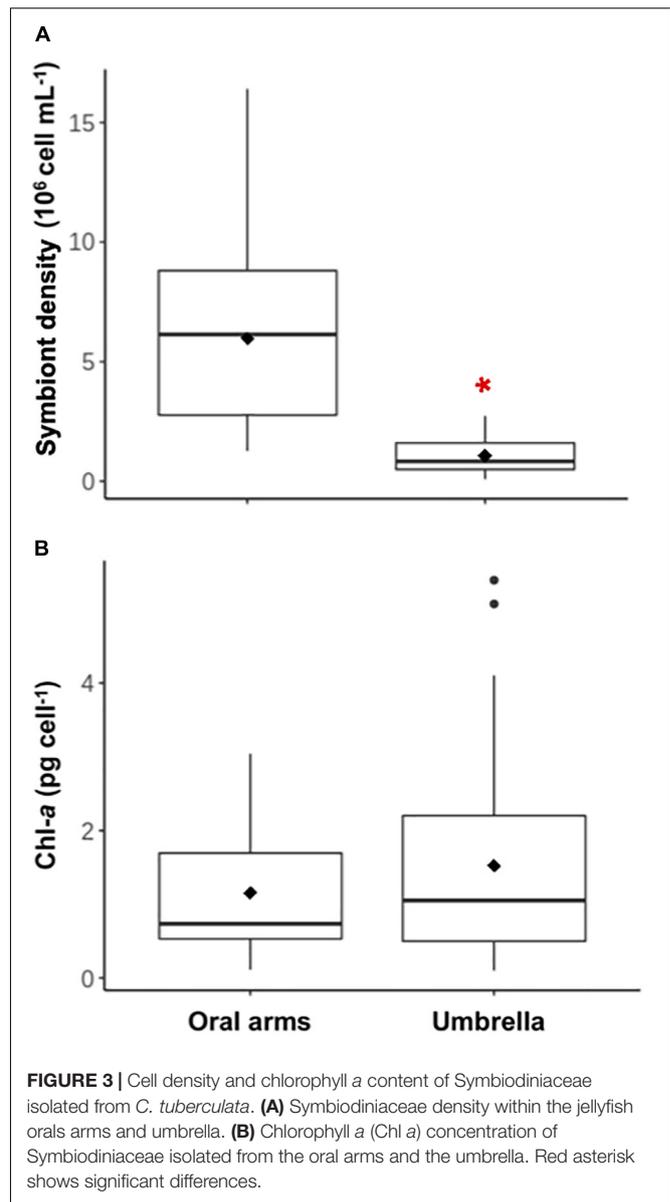
Symbiont Distribution in Hospite

Symbiodiniaceae were heterogeneously distributed within the host body (Figure 3A). We found significantly (Mann-Whitney U -test, $U = 1,789$, $N = 43$, $P < 0.05$) higher algae densities in the OA section ($5.98 \pm 3.58 \cdot 10^6 \text{ cell ml}^{-1}$) than in the UM section ($1.07 \pm 0.72 \cdot 10^6 \text{ cell ml}^{-1}$). However, no relationship or tendency was found between Symbiodiniaceae density and host WM. Cell diameter of freshly isolated algae from the host was $8.71 (\pm 0.97) \mu\text{m}$ ($N = 430$).

Pigment Content

Chlorophyll *a* content and symbiont diameter were also studied separately in both jellyfish body sections, but no significant differences were found (Mann-Whitney U -test, $U = 688$, $N = 43$, $P = 0.474$, Figure 3B). Average Chl *a* content per cell was $1.33 (\pm 0.83) \text{ pg Chl } a \text{ cell}^{-1}$.

HPLC analysis revealed the presence and quantity of 13 different pigments in *C. tuberculata* Symbiodiniaceae (Table 1), that were quantified and expressed as percentage (\pm SD): chlorophyll c_2 (31.25 ± 8.90), chlorophyll *a* (30.12 ± 5.44),



peridinin ($18.47\% \pm 3.41$), diadinoxanthin ($10.10\% \pm 2.81$), fucoxanthin ($5.35\% \pm 6.60$), chlorophyll c_3 ($1.22\% \pm 0.70$), β -carotene ($0.88\% \pm 0.38$), 19'-butanoyloxyfucoxanthin ($0.86\% \pm 0.43$), alloxanthin ($0.72\% \pm 0.62$), 19'-hexanoyloxyfucoxanthin ($0.64\% \pm 0.11$), diadinoxanthin ($0.33\% \pm 0.21$), zeaxanthin and lutein ($0.44\% \pm 0.01$), and chlorophyll *b* ($0.01\% \pm 0.02$).

Cultured Symbiodiniaceae Study

The growth rate of symbionts in culture was $0.52 \cdot 10^6 \text{ cell ml}^{-1} \text{ d}^{-1}$ (Supplementary Figure 1). *In vivo* observation of cultures under light microscopy revealed different phases of Symbiodiniaceae. Cultures obtained from recent isolation of symbionts showed the presence of colored coccoid cells (spherical) (Figures 4A, 5A–C), doublet (dividing)

TABLE 1 | Percentage of pigment content (average% \pm SD) of *Cotylorhiza tuberculata* symbionts (Symbiodiniaceae) from High Performance Liquid Chromatography (HPLC) analyses.

Pigment	%Pigment content (\pm SD)
Chlorophyll c_2	31.25 (\pm 8.90)
Chlorophyll a	30.12 (\pm 5.44)
Peridinin	18.47 (\pm 3.41)
Diadinoxanthin	10.10 (\pm 2.81)
Fucoxanthin	5.35 (\pm 6.60)
Chlorophyll c_3	1.22 (\pm 0.70)
β -carotene	0.88 (\pm 0.38)
19'-butanoyloxyfucoxanthin	0.86 (\pm 0.43)
Alloxanthin	0.72 (\pm 0.62)
19'-hexanoyloxyfucoxanthin	0.64 (\pm 0.11)
Diadinoxanthin	0.33 (\pm 0.21)
Zeaxanthin + Lutein	0.44 (\pm 0.01)
Chlorophyll b	0.01 (\pm 0.02)

$N = 11$ medusae.

(Figures 5D,E), and mastigote (motile zoospore) (Figures 4C, 5F,G). In the most ancient cultures, we only observed coccoid cells (Figures 4A,B), showing the typical mesh-like plastid of Symbiodiniaceae (LaJeunesse et al., 2010), that indicates a reticulated chloroplast located around the entire cell periphery (Figure 4A). Mastigotes were mushroomed-shaped with the hemispherical epistome slightly larger than the hemispherical hyposome (Figures 4C, 5G). The nucleus was in the epistome (Figure 4C). The averaged diameter of coccoid cells was 15.28 (\pm 1.28) μm for the bigger coccoid morphotype ($N = 100$) and 4.83 (\pm 0.678) μm for the smaller morphotype ($N = 100$). This size ranges have been previously observed in vegetative cysts (Stat et al., 2006). It can be inferred that small coccoids might correspond to young vegetative cysts of the symbiont whereas the bigger coccoids represented older cells. Also, doublet cells could be well dividing vegetative cysts giving place to two daughter cells (Figures 5D,E).

In ancient cultures, calcifying biofilm formations were also identified (Figures 6A–D), which were similar to those recently discovered in a diverse range of cultured *Symbiodinium* strains and their inherent bacterial communities (Frommlet et al., 2015). The association between *Symbiodinium* and bacteria produces aragonite microbialites, termed “symbiolites,” which encase adjacent symbiotic algae as endolithic cells (Frommlet et al., 2018). In our Symbiodiniaceae cultures, this calcification formed a biofilm matrix (Figure 6) that was attached to the culture flasks, appearing transparent under light microscopy (Figures 6A,B) and containing numerous coccoids cells and associated microbiota (Figures 6C,D). The matrix was indeed composed by calcium carbonate (see Supplementary Figure 2).

Photosynthesis vs. Irradiance

The photosynthetic behavior of cultured Symbiodiniaceae isolated from *C. tuberculata* was studied by a Photosynthesis-Irradiance (P-I) curve shown in Figure 7. The initial linear part of the P-I curve, where the photosynthetic rate is linearly

proportional to the irradiance (light limited region) or the initial slope of the light saturation curve, determined by α , indicated an averaged photosynthetic efficiency of $3.8 \cdot 10^{-4}$ ($\pm 1.5 \cdot 10^{-4}$) $\mu\text{gC (mg Chl } a)^{-1} \text{ h}^{-1}$ ($\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$). As irradiance increased, the P-I curve showed the typical non-linear tendency reaching a saturation level in which the photosynthetic rate was maximal, P_{max} with a value reaching $5.22 \cdot 10^{-2}$ $\text{mgC (mg Chl } a)^{-1} \text{ h}^{-1}$. The irradiance at the onset of light saturation, I_k , was 142 (± 45) $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, calculated as $I_k = P_{max}/\alpha$. No photoinhibition was observed under 1,200 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$.

Absorbance of Jellyfish Tissue

The UV-VIS absorption spectra of the three sections of the umbrella of *C. tuberculata* intact association are shown in Figure 8. The absorbance of the tissue sections was relatively low at wavelengths >400 nm (Photosynthetically Active Radiation, PAR, 400–700 nm) and high at wavelengths <400 nm (Ultraviolet irradiation, UVR, 290–400 nm). Absorption spectrum of the central dome section (a , $N = 8$), exhibited less accentuated peaks in the UV spectral region and higher absorbance values than tissue b and c . In this section, a high absorbance was also observed in the PAR region, where smooth peaks of 580 and 630 nm approximately were evident. Clear section (b , $N = 11$) presented two absorption peaks at 270 and 330 nm approximately, but the absorption in the visible light spectrum was higher than in section c . Umbrella edge (c , $N = 12$), also showed two absorption peaks at 270 and 330 nm approximately. However, the absorption was minimal in the visible light spectrum (400–700 nm).

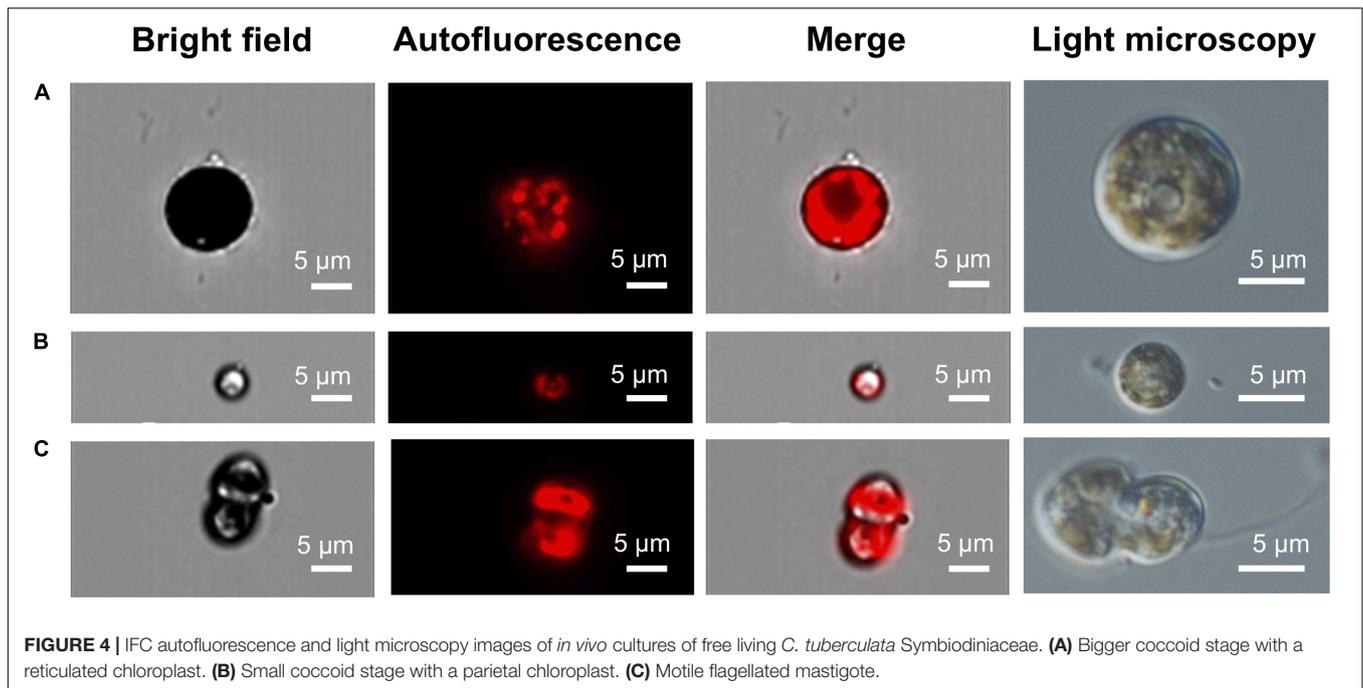
The mean thickness of the umbrella tissue sections of 4 individuals of *C. tuberculata* (mean umbrella diameter, 20 ± 4.78 cm) were 13 (± 2.89), 22 (± 3.08), and 5 (± 1.65) mm for central dome (a), clear section (b), and umbrella edge (c), respectively. In the laboratory, light attenuation provided by each jellyfish tissue section calculated as percentage of the surface irradiance (100%) was a 75% by central dome, 41% by clear section and 14% by umbrella edge.

DISCUSSION

Role of the Jellyfish in the Symbiotic Association Alga-Cnidarian

The light environment surrounding Symbiodiniaceae is a key factor to understand photosymbiosis. The productivity of the symbiosis depends on the environmental light quantity and quality, in other words, the photon flux and the spectral composition of light reaching the algae (Roth, 2014). Therefore, the depth at which the jellyfish lives and the characteristic of the surrounding seawater, as well as the shade and protection provided by the medusa body itself constitute an important aspect of the Symbiodiniaceae-jellyfish symbiosis.

The spectral composition of the light field inside the medusa tissue might be a key factor regulating photosynthesis of the symbiont. The light field available for symbionts living within the medusa body depends on the optical properties of the host



tissues and differs from the incident environmental irradiance due to the scattering and absorption, as demonstrated in scleractinian corals (Kühl et al., 1995). This study shows for the first time the absorption spectrum of a zooxanthellate jellyfish tissue *in vivo*. The light absorption of *C. tuberculata* umbrella tissue mostly absorbs on two ultraviolet absorption peaks at approximately 270 and 330 nm. The absorbance of the three umbrella tissue sections (umbrellar edge, clear section, and central section) was high at ultraviolet wavelengths (below 400 nm), indicating that medusae tissue plays an important role protecting Symbiodiniaceae from UV radiation. These results are consistent with those by Higuchi et al. (2010), who found two similar absorption peaks in the UV spectrum using homogenate host tissue extract of the symbiotic coral *Galaxea fascicularis* and suggested the formation of UV-absorbing compounds in their tissues. Ultraviolet radiation (UVR) is the most damaging portion of the light spectrum, known to have negative effects on physiological and cellular processes, damaging the host tissue and the algae photosynthetic apparatus (Arai, 1997). These high-energy wavelengths cause damage in biologically relevant molecules, chloroplast and photosynthetic apparatus, causing photoinhibition, which leads to the generation of multiple reactive oxygen species (ROS) by the symbionts (Weis, 2008). The effects of UVR have been studied in several Symbiodiniaceae species living in symbiosis with corals and anemones (Lesser and Shick, 1990; Lesser and Lewis, 1996) and, to a lesser extent, in jellyfish (e.g., the scyphozoan *Cassiopea* sp. Klein, 2016). Under UVR exposition, symbionts of *C. xamachana* exposed to UVR produced microsporine-like amino acids (MAAs) (Banaszak and Trench, 1995) which are UV-absorbing compounds that act as UV sunscreen and confer protection during heat stress (Banaszak and Lesser, 2009). *C. xamachana* absorption spectra at UV

wavelengths was characterized by a maximum peak at 324 nm and their freshly isolated symbionts at 338 nm (Banaszak and Trench, 1995) similar to those found here for *C. tuberculata* (330 nm), which may be indicative of the presence of MAAs in this jellyfish tissues. However, despite MAAs accumulate in the host tissues, it is still unclear the symbiotic partner that produces these molecules (Shinzato et al., 2011).

The structure of *C. tuberculata* umbrella provides different light environments. The dark-colored central dome showed a high absorbance not only in the UV region but also in the PAR region (580–630 nm). Furthermore, light attenuation provided by this tissue section reaches 75% of the environmental irradiance, which may eventually result in light limitation for symbiont photosynthetic activity. Light attenuation provided by host white tissue in the thicker part of the bell was 44%. Symbionts are especially numerous along the radial canals of the gastrovascular system (Kikinger, 1992) in the central dome of the umbrella that provides UVR protection. Finally, the umbrella edge is the thinner part of the jellyfish bell, providing 14% of light attenuation. Here, the absorption is elevated in the UVR region of the spectrum and remains very low in the PAR region, suggesting that no light limitation occurs here, but the light conditions in this region might be damaging the algae. However, no Symbiodiniaceae were found within this tissue.

Light reaching Symbiodiniaceae in hospite will also depend on the host position in the water column, the characteristics of the surrounding seawater and the incident environmental irradiance. Zooxanthellate jellyfish are known to migrate vertically to mitigate UV damage or photoinhibition of their photosymbionts, as well as to maximize their photosynthetic rates (Cimino et al., 2018). *C. tuberculata* specimens used in this work occur in the Mar Menor, a shallow coastal lagoon (4 m depth on average)

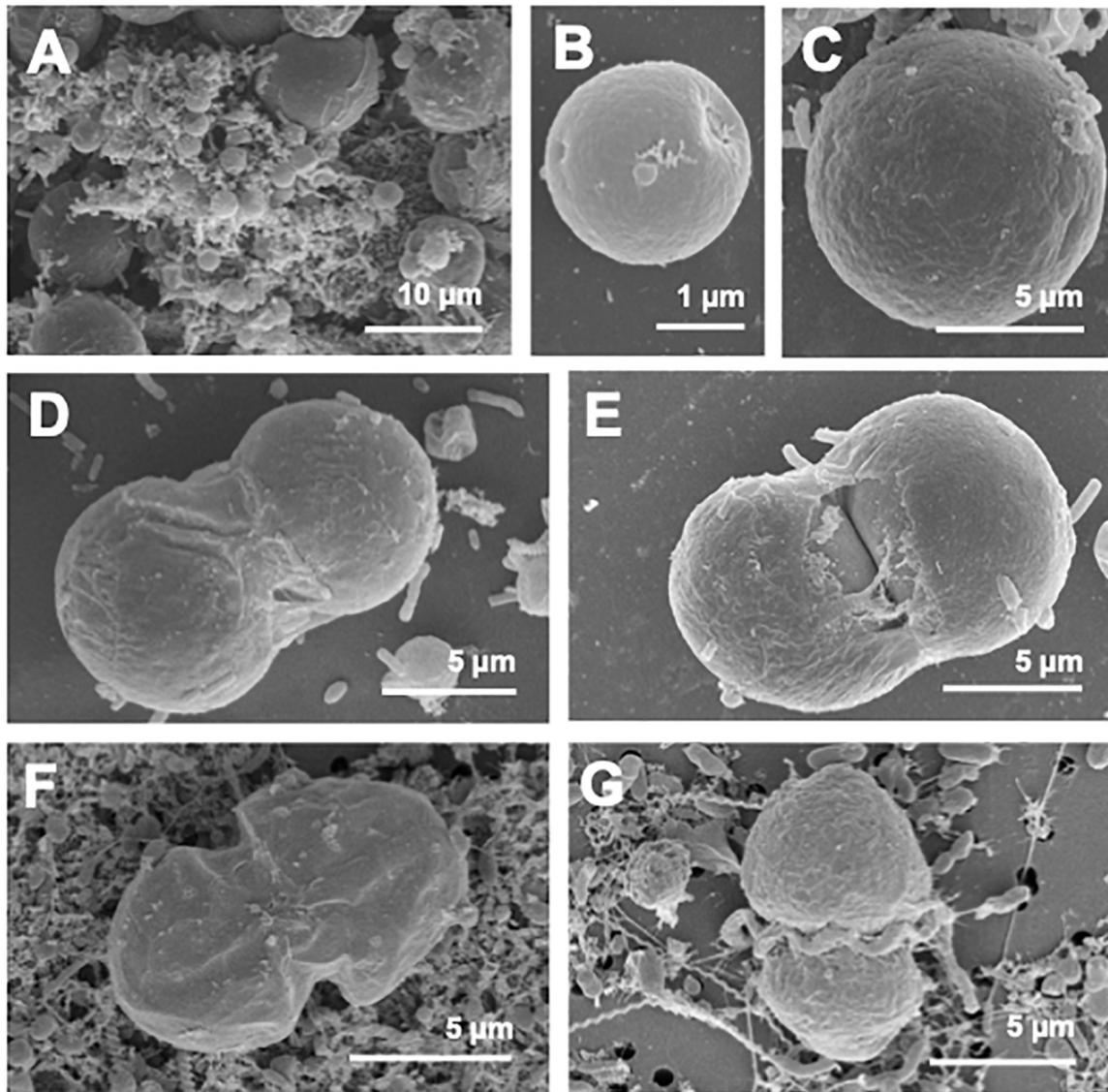


FIGURE 5 | SEM micrographs of different cellular stages observed in Symbiodiniaceae cultures. **(A)** *C. tuberculata* symbionts of both sizes. **(B)** Small coccoid cells observed in cultures. **(C)** Bigger coccoid stage. **(D,E)** Doublet, two different phases of bigger coccoid cells undergoing mitosis. **(F,G)** Motile flagellated mastigote.

(Pérez-Ruzafa et al., 2005). In late summer and early autumn, when the pelagic phase of the jellyfish thrive, the lagoon turns mesotrophic reaching high chlorophyll levels (Pérez-Ruzafa et al., 2005) and irradiance measured at the bottom of the lagoon remains lower than the rest of the year (Terrados and Ros, 1991). *C. tuberculata* moves in the water column between 1 and 2 m depth (Kikinger, 1992). The calculated light intensity reaching the medusa at 1 and 2 m depth in the Mar Menor was 2,681 and 1,797 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively. Given that the jellyfish umbrella tissue where most Symbiodiniaceae concentrate, attenuates the surrounding irradiation by 41%, the light intensity received by the symbionts at 1 m of the surface would be 1,100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ and 736 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, at 2 m depth (Figure 9). The study of the

photosynthesis-irradiance of *C. tuberculata* symbionts revealed no photoinhibition at irradiances below 1,200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, suggesting that Symbiodiniaceae inside their host are protected from the elevated irradiances reached in the surface water. Therefore, the great importance of the jellyfish tissue attenuating the elevated environmental irradiance in the upper water column and absorbing damaging UVR is evidenced. The vertical distribution of zooxanthellate jellyfish occupying a specific depth range to obtain the adequate oxygen for respiration and light for photosynthesis has been previously studied in other zooxanthellate jellyfish (Hamner et al., 1982; Muscatine and Marian, 1982; Dawson and Hamner, 2003). *C. tuberculata* optimal depth in the Mar Menor may be however influenced by light penetration, water turbidity, seasonal light intensity, and

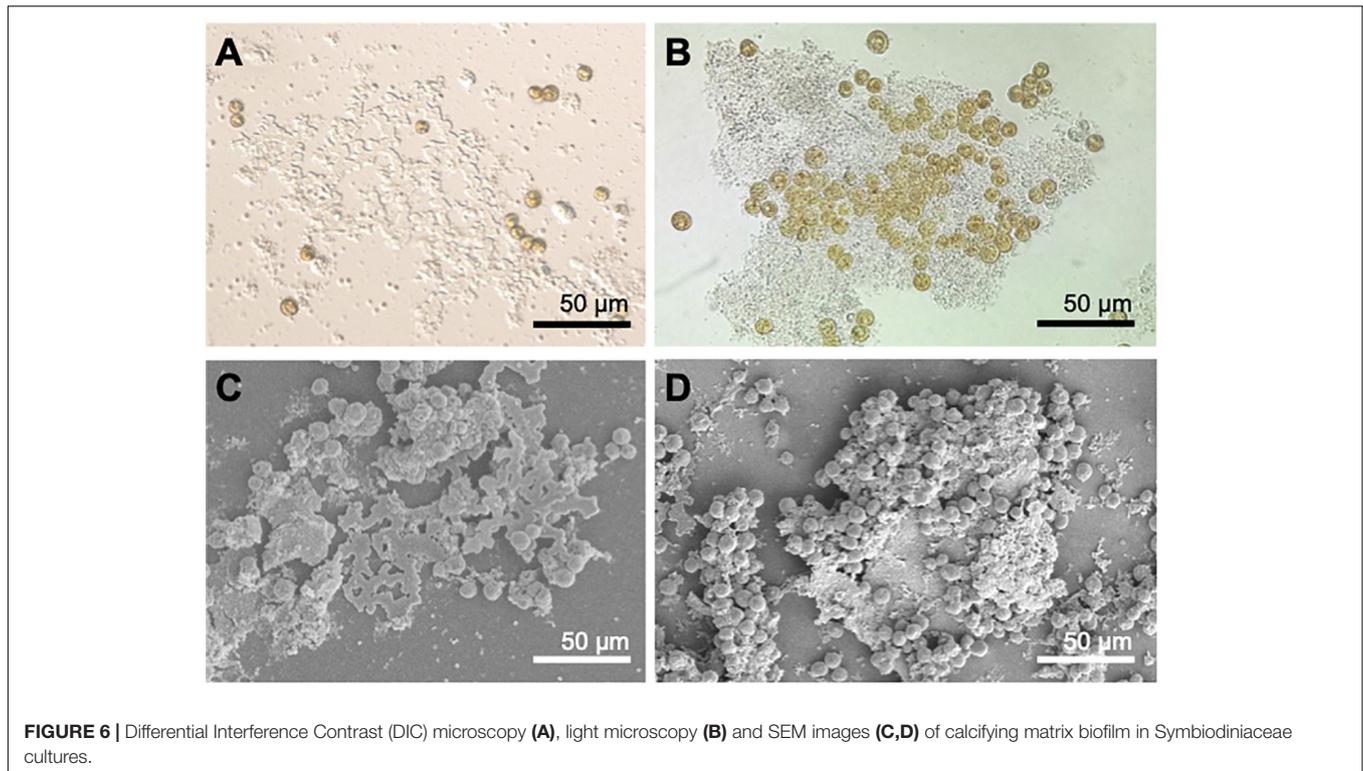


FIGURE 6 | Differential Interference Contrast (DIC) microscopy (A), light microscopy (B) and SEM images (C,D) of calcifying matrix biofilm in Symbiodiniaceae cultures.

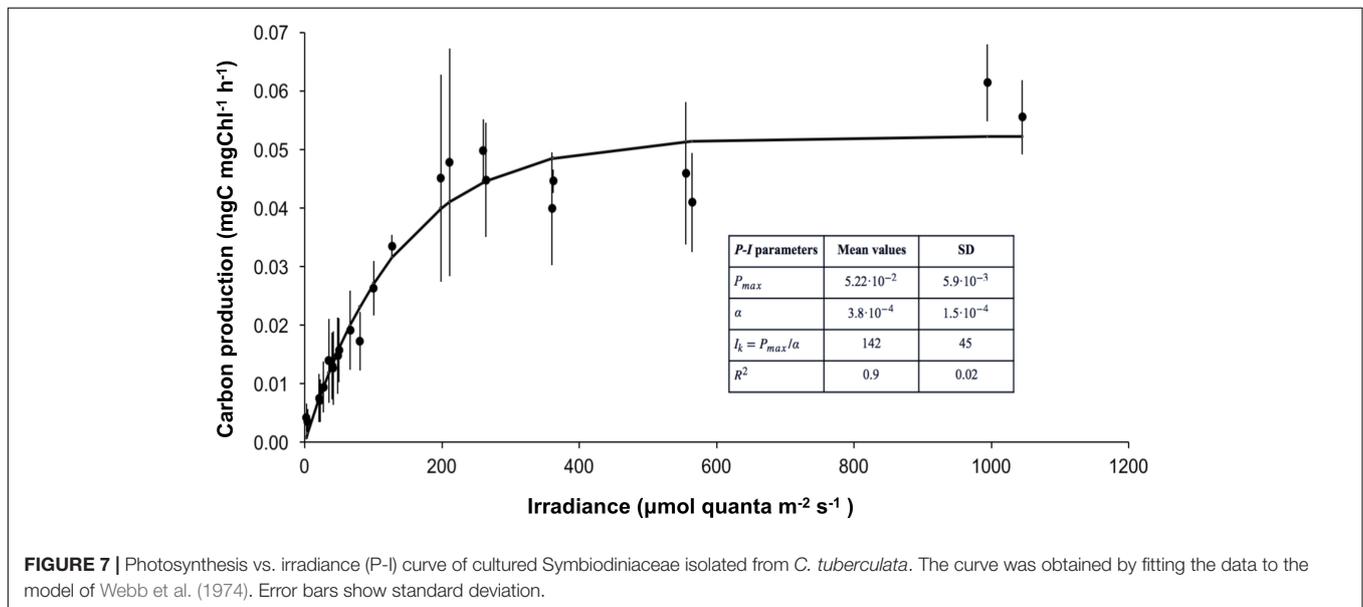


FIGURE 7 | Photosynthesis vs. irradiance (P-I) curve of cultured Symbiodiniaceae isolated from *C. tuberculata*. The curve was obtained by fitting the data to the model of Webb et al. (1974). Error bars show standard deviation.

cloud cover. Hence, the jellyfish will respond to changes in the vertical water column by choosing a depth to match their optimal physiological tolerance and maximize photosynthesis.

Symbiont Distribution Within the Jellyfish Body

The abundance of Symbiodiniaceae is an important feature of the symbiosis because it directly affects the amount of

oxygen produced by photosynthesis. In zooxanthellate jellyfish the density of symbionts is affected by their complex life-cycle. In the case of *C. tuberculata* symbionts are acquired during the polyp stage (Kikinger, 1992; Verde and McCloskey, 1998; Astorga et al., 2012), reaching at this point, algal cell densities between 100 and 300 cells per mm² (Enrique-Navarro et al., 2021). During strobilation, the algae multiply in the oral region of the polyp where the ephyrae are formed (Ludwig, 1969) hence, releasing zooxanthellate ephyrae (Kikinger, 1992). On the other hand,

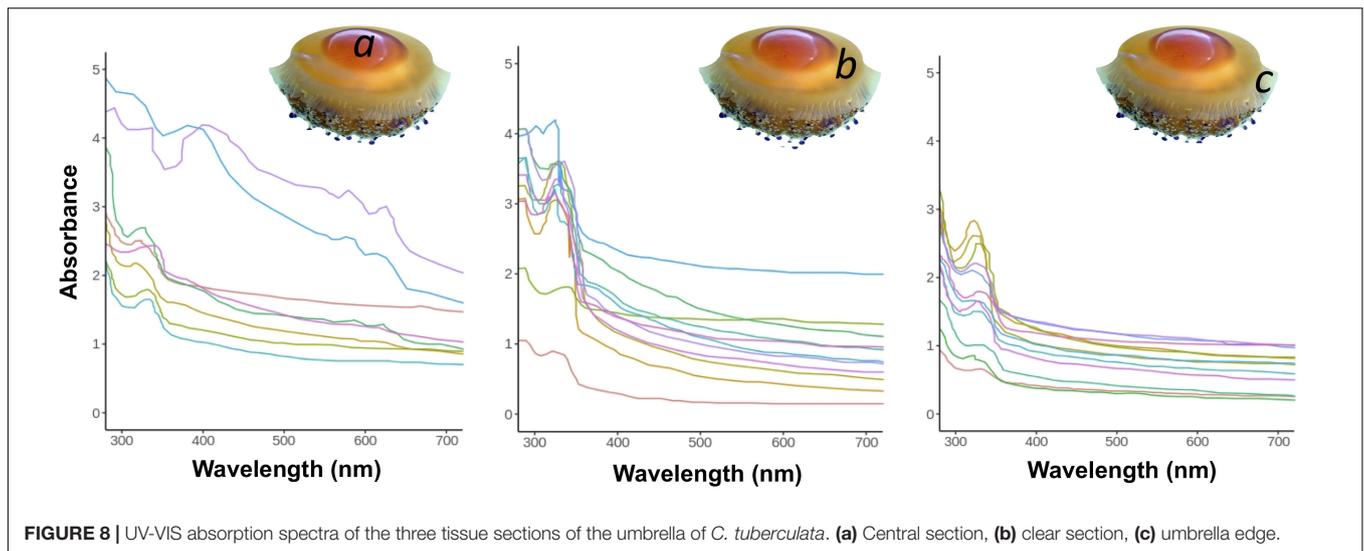


FIGURE 8 | UV-VIS absorption spectra of the three tissue sections of the umbrella of *C. tuberculata*. **(a)** Central section, **(b)** clear section, **(c)** umbrella edge.

during medusae growth the abundance of symbionts remains constant or decreases slightly in most species down to the order of 10^6 cells g^{-1} wet mass (Muscatine et al., 1986; Kremer et al., 1990; Verde and McCloskey, 1998).

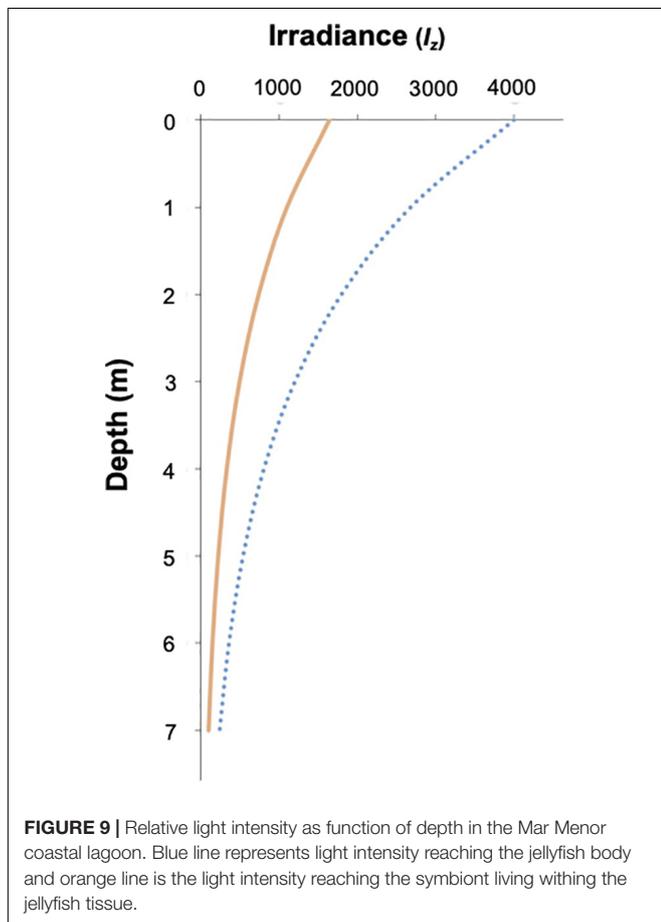


FIGURE 9 | Relative light intensity as function of depth in the Mar Menor coastal lagoon. Blue line represents light intensity reaching the jellyfish body and orange line is the light intensity reaching the symbiont living within the jellyfish tissue.

Our results show that symbiotic algae were heterogeneously distributed within the medusa body, being 5.5 fold denser in the oral arms than in the umbrella (**Table 2**). This heterogeneity might be a consequence of the different body parts roles, functions and morphologies. The jellyfish oral arms act as an uptake organ, thus being a nutrient-rich environment for symbionts nutrition and consequently, for algal primary production (Muscatine et al., 1986). In addition, oral arms and tentacles have a sinuous folding morphology that provides a large area to host microalgae. On the other hand, the bell has a well-developed muscle cell organization to allow active contraction and movements during medusa locomotion. In this part of the animal, the symbionts are concentrated in the radial canals of the gastrovascular system (Kikinger, 1992). The elevated zinc levels measured in *C. tuberculata* oral arms (4 times higher than umbrella) is also an indication of the higher presence of symbionts in these organs, as zinc is a key element of the enzyme carbonic anhydrase in algae (Furla et al., 2011). In *C. tuberculata*, the symbiont density was independent of the body size, as previously reported in *Mastigias* sp. (Muscatine and Marian, 1982; Muscatine et al., 1986) and cell density values were similar to those observed in *L. unguiculata* (Kremer et al., 1990) and *C. xamachana* (Verde and McCloskey, 1998), in which the symbionts densities were also two to threefold higher in the oral arms than in the bell (Blanquet et al., 1979; Estes et al., 2003; **Table 2**). This finding is also consistent with the larger values of C measured in the oral arms of *C. tuberculata*. Overall, jellyfishes are characterized by high water (>95%) and low carbon (<1% of WM) contents, which enables them to increase their size in a much larger way than non-gelatinous animals with an equivalent C content (Pitt et al., 2013). This composition offers many adaptive advantages (Acuña et al., 2011). In our study, biochemical composition of *C. tuberculata* in terms of carbon and nitrogen was quantitatively similar than that in non-symbiotic (*Aurelia aurita*, *Chrysaora fuscescens*, *Chrysaora hysoscella*, *Pelagia noctiluca*, and *Rhizostoma pulmo*) and symbiotic scyphozoans (*Cassiopea xamachana*, *Cyanea*

TABLE 2 | Comparison of the Symbiodiniaceae parameters of chlorophyll *a* concentration, cell density, and diameter of 4 symbiotic jellyfish: *Cotylorhiza tuberculata*, *Cassiopea xamachana*, *Linuche unguiculata*, and *Mastigias* sp.

Species	Chl <i>a</i> concentration (pg cell ⁻¹)	Symbiodiniaceae density (× 10 ⁶ cell g ⁻¹)	Cell diameter (μm)	References
<i>Cotylorhiza tuberculata</i>	1.33	8.45	8.71 (in hospite)	Present study
<i>Cassiopea xamachana</i>	1.00–1.45 (summer) 2.21 (winter)	9.6–4.9	8.64 (summer) 9.08 (winter)	1, 2, 3
<i>Linuche unguiculata</i>	2.00–2.10	4.5	8.9	4, 5
<i>Mastigias</i> sp.	2.00	–	9.85 (small specimens) 8.25 (large specimens)	6

1. Vodenichar (1995), 2. Verde and McCloskey (1998), 3. Estes et al. (2003), 4. Kremer et al. (1990), 5. Wilkerson and Kremer (1992), 6. McCloskey et al. (1994).

capillata, and *Mastigias* sp.) whose overall C:N ratio falls within the range comprised between 3.2 and 4.5 (Table 3). Even though comparison with the C:N ratios obtained in different parts of the considered jellyfishes is not possible, it is reasonable to assume that the oral arms in zooxanthellate jellyfish would contain a higher amount of carbon than the bell does. This carbon would be the result of the own animal metabolism plus that produced during photosynthesis due to a high accumulation of symbionts. Similarly, this could be the situation encountered in the tentacles that host a higher algal density. On the contrary, the bell or umbrella, with a lower concentration of symbionts, would contain a lower carbon content than the arms but a similar nitrogen proportion that causes in turn, a similar C:N ratio between both parts of the medusa.

Symbiodiniaceae Pigments

Photosynthetic dinoflagellates including Symbiodiniaceae have plastids derived from red algae. The most representative photosynthetic pigments in *C. tuberculata* symbionts are found to be chlorophyll *a*, chlorophyll *c*₂, and peridinin, the primary photosynthetic pigments of Symbiodiniaceae (Hennige et al., 2009; Roth, 2014). Host-specialized dinoflagellates have different light-harvesting complexes to capture light: the (1) the thylakoid membrane-bound chlorophyll *a*–chlorophyll *c*₂–peridinin-protein-complex (acpPC) and (2) the water-soluble peridinin–chlorophyll *a* protein (PCP) (Iglesias-Prieto et al., 1991, 1993). The chlorophylls absorb high-energy blue light (~430–460 nm), but chlorophyll *a* absorbs also red light (~680 nm) (Bricaud et al., 2004). Chl *a* concentration per cell (1.33 ± 0.83 pg cell⁻¹) of *C. tuberculata* symbionts was consistent with those reported for other jellyfish symbionts (Table 2). Similar Chl *a* concentration was found in *C. xamachana* symbionts (Vodenichar, 1995; Verde and McCloskey, 1998) as well as, *L. unguiculata* and *Mastigias* sp. symbionts (Kremer et al., 1990; Wilkerson and Kremer, 1992; McCloskey et al., 1994). Peridinin, which constitutes the typical biomarker pigment on dinoflagellates (Barlow et al., 1993; Jeffrey et al., 2011), has

TABLE 3 | Comparison of biochemical C and N composition and C:N ratio expressed as percentage of dry mass (%DM) of symbiotic and non-symbiotic scyphozoan jellyfish: *Cotylorhiza tuberculata*, *Aurelia aurita*, *Cyanea capillata*, *Chrysaora fuscescens*, *Chrysaora hysoscella*, *Cassiopea xamachana*, *Mastigias* sp., *Pelagia noctiluca*, and *Rhizostoma pulmo*.

Species	C (%)	N (%)	C:N ratio	References
<i>Cotylorhiza tuberculata</i>	7.7 (OA) 4.9 (UM)	1.7 (OA) 1.3 (UM)	4.5 (OA) 3.7 (UM)	Present study
<i>Aurelia aurita</i>	4.3	1.3	3.2	1
<i>Cyanea capillata</i>	10	2.5	4.0	2
<i>Chrysaora fuscescens</i>	7.0	2.0	3.5	3
<i>Chrysaora hysoscella</i>	5.2	1.4	4.3	4
<i>Cassiopea xamachana</i>	4.3	1.3	3.3	5
<i>Mastigias</i> sp.	1.6	0.5	3.3	6, 7
<i>Pelagia noctiluca</i>	6.5	1.8	4.0	4
<i>Rhizostoma pulmo</i>	8.5	2.2	4.5	4

1. Larson (1986), 2. Bailey et al. (1995), 3. Shenker (1985), 4. Kogovšek et al. (2014), 5. Verde and McCloskey (1998), 6. McCloskey et al. (1994); 7. Ikeda (2014).

a maximum absorption of blue-green light (~450–550 nm), expanding the range of photosynthetically usable light of Symbiodiniaceae (Bricaud et al., 2004). Symbiodiniaceae in hospite and cultured photoacclimate to different light conditions by modifying the amount of chlorophyll *a*, chlorophyll *c*₂, and peridinin per cell. In symbiosis, the pigment ratios are maintained. However, in culture, the proportion of pigments can be modified (Iglesias-Prieto and Trench, 1994; Hennige et al., 2009). This discrepancy between dinoflagellate symbionts in hospite and cultured suggest that the host modulates the light environment of Symbiodiniaceae in symbiosis (Roth et al., 2010).

Carotenoids (carotenes and xanthophylls) are accessory pigments synthesized by photosynthetic organisms. Symbiodiniaceae hosted by *C. tuberculata* synthesizes β-carotene and xanthophylls peridinin, diadinoxanthin, and diatoxanthin (Table 1). Carotenoids are part of the photoprotective mechanisms of the photosynthetic organisms (Jeffrey and Haxo, 1968) and have a variety of roles, acting as accessory light-harvesting pigments, components of the light-harvesting complexes, antioxidants, and sinks for excess energy (Roth, 2014). Under high light conditions, the xanthophyll cycle is activated and diadinoxanthin is de-epoxidated to diatoxanthin protecting the photosynthetic apparatus against the excess excitation and the consequent oxidative damage (Lavaud et al., 2002). Symbiodiniaceae can increase the capacity for photoprotection by adjusting the amounts of carotenoids (Hennige et al., 2009). Therefore, higher contents of diadinoxanthin in *C. tuberculata* symbionts may suggest the photoprotection provided by the medusa tissue.

The occurrence of zeaxanthin-lutein and chlorophyll *b*, present at a very low percentage, may indicate the occurrence of other types of photosynthetic organisms (such as microscopic endolithic algae) present in the samples by

accidental contamination (Torres-Pérez et al., 2015) or the presence of photosynthetic bacteria hosted in the gastric cavity of *C. tuberculata* (Viver et al., 2017). Fucoxanthin and 9'-hexanoyloxyfucoxanthin, also found in very low quantity, are not characteristic of dinoflagellates, but it has been previously identified in this group (Jeffrey et al., 2011).

Symbiodiniaceae *ex hospite*

The growth rate of symbiotic algae is regulated by host factors and growth (Muscatine et al., 1986). Once released from the host regulation factors, cultured Symbiodiniaceae rapidly adapt to free-living conditions (D'Elia and Domotor, 1984). Furthermore, increased nutrient availability outside the host body (where N and P is limited) allows symbionts to reach higher growth rates (Miller et al., 1989; Stoner et al., 2011). The growth rate of *C. tuberculata* symbionts in culture was consistent with other cultured dinoflagellates isolated from jellyfish hosts (Fitt and Trench, 1983; D'Elia and Domotor, 1984; Taguchi and Kinzie, 2001) and was similar to those of free-living dinoflagellates (Tang, 1996). However, the frequency of mitosis or growth rate of endosymbiotic algae remains much slower (0.05 and 0.1 d⁻¹, respectively, in Wilkerson et al., 1983; Muscatine et al., 1986) than those of *ex hospite* Symbiodiniaceae.

In our study, the two known associates of *C. tuberculata* were not genetically distinguished. However, previous genetic analyses on individuals from the Mar Menor (Raspor Dall'Olio, 2016), revealed the presence of the Symbiodiniaceae species *Philozoon medusarum* (LaJeunesse et al., 2021) and *Breviolum psygmophilum* (LaJeunesse et al., 2012; LaJeunesse et al., 2018). Even though *C. tuberculata* may occur symbiotically with distinct Symbiodiniaceae species, its co-occurrence in symbiosis with different species simultaneously has not been demonstrated yet (Raspor Dall'Olio, 2016).

The Symbiodiniaceae life cycle is composed of different stages: (i) coccoid cells are the dominant stage seen inside the host (**Supplementary Figure 3**), (ii) dividing doublet are coccoid cells undergoing mitosis, (iii) motile mastigotes only occur in free living conditions. Coccoid vegetative cells presented a reticulated chloroplast typical of Symbiodiniaceae (LaJeunesse et al., 2010), the mesh-like plastid is located around the entire cell periphery allowing symbionts to receive photons from any directions. The morphological adaptation of the coccoid cell plastid help in maximizing light absorption efficiency in hospite (LaJeunesse et al., 2010). Vegetative coccoid cells were associated with bacteria in our cultures forming a calcifying bacterial-algal biofilm (**Figure 6**). This calcification process has been described in *ex hospite* symbionts of cnidarian hosts (corals and sea anemones) and it is driven by the photosynthetic activity of Symbiodiniaceae but, requires naturally associated bacterial communities to occur (Frommlet et al., 2015, 2018). The process is known as "Photosynthetic Induced Carbonate Precipitation" and is influenced by microbial activity on calcification (Frommlet et al., 2018). Motile flagellated cells or mastigotes were present in cultures during the first month after isolation from the host. Cells with mastigotes possessed seven latitudinal series of amphiesmal vesicles as described by LaJeunesse et al. (2018) and, longitudinal and transverse flagella.

CONCLUSION

In this study, we examined the characteristics of the symbiosis between the bloom-forming scyphozoan jellyfish *Cotylorhiza tuberculata* and their photosynthetic partners of the family Symbiodiniaceae. The host-specialized dinoflagellates are heterogeneously distributed within the host body being more concentrated in the medusa oral arms as a consequence of the tissue role, their high metabolic activity and their sinuous folding. The C:N ratio were also elevated in the oral arms indicating lower protein content. The study of the symbionts in hospite revealed no significant differences in size neither chlorophyll *a* concentration within the oral arms and the umbrella. Pigment composition in symbionts included typical Symbiodiniaceae light-harvesting pigments (chlorophyll *a*, chlorophyll *c*₂, and peridinin) as well as carotenoids (β-carotene, xanthophylls peridinin, diadinoxanthin, and diatoxanthin), involved in the protection of the photosynthetic apparatus. Furthermore, cultured Symbiodiniaceae behave as free-living dinoflagellates exhibiting elevated growth rates and developing motile mastigotes cells. Symbionts acclimates to a free-living environment acting as a planktonic dinoflagellate when released from host regulation. The morphology of Symbiodiniaceae in culture revealed the presence of vegetative coccoid cells that divided by mitosis (doublet) and motile mastigotes. In addition, we found the typical calcifying matrix synthesized by Symbiodiniaceae and their microbiota in most algal cultures. Finally, the study of the photosymbiosis revealed the light conditions of Symbiodiniaceae in hospite and the protective role played by medusa body. Jellyfish tissue protects the symbionts from the negative effects of ultraviolet radiation and attenuates the elevated environmental light intensity avoiding damages in the algae photosynthetic apparatus. The presence of a dense Symbiodiniaceae population and the protection to UVR provided by medusae tissue maintaining photosymbionts in optimal light conditions to photosynthesis may be a reason added to explain the population success of *Cotylorhiza tuberculata*.

Despite 20–25% of Scyphozoa being zooxanthellate, basic information on the biology and ecology of most species is still lacking. Therefore, our work may contribute to the scientific knowledge of zooxanthellate jellyfish ecology. Future research on the biology of less studied symbiotic jellyfish and their host-specialized dinoflagellates using more a systematic approach and more recent methods are needed. Most of our knowledge about photosymbiosis comes from scleractinian corals. Consequently, interesting research questions and ideas for future research could be also derived from the extensive knowledge and experience provided by the scientific field of symbiotic corals.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

AE-N: data acquisition, data analysis, and writing original draft and editing. EH: supervision, reviewing, and funding acquisition. VF-P and AB: data acquisition, data analysis, and reviewing. GN: data analysis and reviewing. JR and AM: supervision and reviewing. LP: supervision, reviewing, data acquisition, and funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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