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Cytoplasmic UV-R Absorption in an Integumentary Matrix (tunic) of Photosymbiotic Ascidian Colonies

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Noburu Sensui and Euichi Hirose (2018) In didemnid ascidians with cyanobacterial symbionts, the tunic has a specific peak absorbing ultraviolet radiation (UV-R) due to the presence of ultraviolet (UV)-absorbing compounds, which probably include mycosporine-like amino acids (MAAs). The UV-R absorbing tunic is supposed to protect the symbionts in the common cloacal cavity of the host colony. The histological distribution of UV-R absorption in the tunic was examined using a UV light microscope equipped with a digital camera, from which the low-pass filter of the UV-sensitive image sensor was removed. The cell peripheries of tunic bladder cells and cell-like objects were visualized with the trans-illumination of UV light, indicating UV-R absorption at that site. In contrast, tunic matrix and vacuolar content of tunic bladder cells appeared to lack of UV-R absorption, allowing damaging wavelengths to penetrate. Accordingly, UV-absorbing compounds are expected to be contained in the cytoplasmic matrix of tunic bladder cells and possibly other types of tunic cells.

Key words: Colonial ascidian, Cyanobacterial symbiosis, *Prochloron*, Tunic, UV microscopy.

BACKGROUND

Symbiosis between ascidians and cyanobacteria is the only life-long algal symbiosis system in chordates (reviewed in Hirose et al. 2009). The host ascidians are always colonial and are distributed in subtropical and tropical waters (*e.g.*, Kott 2001; Monniot and Monniot 2001), as the symbionts are sensitive to low water temperature (Dionisio-Sese et al. 2001). With the exception of some species that harbor algal cells on the colony surface, the host ascidians establish obligate symbionts, which are transferred from generation to generation (reviewed in Hirose 2015). Although there is an argument about the translocation of nutrition between host ascidians and algal symbionts (Hirose and Maruyama 2004), symbiotic cyanobacteria and bacteria produce bioactive secondary metabolites that are important for the survival of the host (reviewed in Schmidt 2015).

Prochloron is the most common and best-investigated cyanobacteria among ascidian symbionts. Kühl et al. (2012) described the microenvironment and physiology of *Prochloron* inhabiting the cloacal cavity of the host colony. The draft genome of *Prochloron* encodes metabolic genes that are apparently sufficient to proliferate in a free-living stage (Donia et al. 2011), but a sustainable culture of *Prochloron* or other cyanobacterial symbionts has not been reported to

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date. Therefore, the host ascidians likely provide an essential microenvironment for the symbionts to proliferate, and the preparation of a suitable environment for the symbionts is an important factor for maintaining the symbiosis system.

Symbiotic cyanobacteria need photosynthetically active radiation (PAR: 400-700 nm), but solar radiation also includes harmful ultraviolet radiation (UV-R: 280-400 nm). The water attenuates UV-R, but organisms living close to the water surface are exposed to significant levels of UV-R particularly environments in which the waters are transparent (*e.g.*, Piazena et al. 2002; Tedetti and Semperv 2006). Field experiments demonstrated that UV-R damages biological molecules (DNA, proteins, lipids, etc.) and leads embryos to develop abnormally (Lamare et al. 2007; Hernández et al. 2010; Lister et al. 2010; Cubillos et al. 2015). Therefore, protection from UV-R may be one of the essential factors for symbionts to maintain stable colonization in the host. Ascidians are sessile organisms and often occur in shaded habitats that are protected from direct solar radiation. For instance, the solitary ascidian *Corella inflata* is vulnerable to UV-R, so it is restricted to shaded habitats (Bingham and Reyns 1999; Bingham and Reitzel 2000). Some species protect the body with a pigmented tunic, which is an integumentary, extracellular matrix of the ascidians (*e.g.*, Hirose 1999). These measures of photoprotection are not suitable for ascidians harboring photosymbionts because enough amount PAR needs to penetrate the tissues surrounding the symbionts.

Tunic absorption spectra in some photosymbiotic ascidians have UV-R absorbing peaks that have been never found in the non-photosymbiotic tunicates so far examined (Hirose et al. 2004; Hirose et al. 2015; Kakiuchida et al. 2017; Sakai et al. 2018). Therefore, the absorption specific to UV-R in the tunic is supposed to be an adaptation to protect the symbiotic cyanobacteria in the host colony from UV-R. Some mycosporine-like amino acids (MAAs) that are also ultraviolet (UV)-absorbing compounds have been isolated from the ascidian-cyanobacteria symbiosis systems, such as mycosporine-glycine (λ_{max} = 310 nm), palythine (λ_{max} = 320 nm), porphyra-334 (λ_{max} = 334 nm), shinorine (λ_{max} = 334 nm) (Lesser and Stochaj 1990; Dunlap and Yamamoto 1995; Dionisio-Sese et al. 1997; Hirose et al. 2004; Hirabayashi et al. 2006). For example, a previous study carried out in the photosymbiotic ascidian *Didemnum molle* showed that the contents of MAAs were significantly different among specimens from different depths (Hirose et al. 2006). MAAs have absorption peaks within the range of UV-R and no PAR absorption, which is consistent with the absorption spectra of the tunic in the photosymbiotic ascidians. These UV-absorbing compounds presumably protect the organisms from UV-R and oxidative stress, and they have been found in varieties of organisms all around the world, *e.g.*, bacteria, cyanobacteria, fungi, lichens, microalgae, macroalgae, and marine animals (reviewed in Karentz 2001; Shick and Dunlap 2002; Carreto and Carignan 2011). Although metazoans do not synthesize MAAs because of their lack of a biochemical pathway, Sick et al. (2002) suggested the possibility of MAA biosynthesis in some sea anemones. Recently, an MAA biosynthetic gene cluster was identified in cyanobacteria (Balskus and Walsh 2010) and the set of genes required for the biosynthesis of shinorine was found in the genomes of the scleractinian coral *Acropora digitifera* and the sea anemone *Nematostella vectensis*, indicating that some anthozoans synthesize MAAs *de novo* (Shinzato et al. 2011). Since the *Prochloron* genome encodes genes involved in the biosynthesis of MAAs (Donia et al. 2011), most MAAs in the host tunic likely originate from their cyanobacterial symbionts. These genes have never been reported in ascidians to date.

As an integumentary tissue, the ascidian tunic is unique among metazoans. The tunic matrix is comprised of cellulose fibrils and free cells called tunic cells that are distributed in this extracellular matrix, *i.e.*, outside of the epithelium (reviewed in Hirose 2009). To specify the histological distribution of UV-absorbing compounds in the tunic, Maruyama et al. (2003) examined cross-sections of unfixed colonies of a *Diplosoma* sp. using a UV light microscope equipped with a film-based camera. Absorption of UV-R appeared to be localized in the cytoplasm but not vacuolar lumen of tunic bladder cells, a cell-type of tunic cells, in the UV light micrographs. However, the micrographs taken with an 20x objective lens were not clear enough to specify the cytological distribution. Since the focus positions were slightly different between the photographs with visible light and UV light, it was difficult to obtain clear images at a high magnification with a film-based camera. In order to understand how photoprotective compounds are distributed in the tunic of two photo-symbiotic ascidians (*Diplosoma virens* and *D. simile*), images at high magnification were taken in this study using a digital camera mounted on a

UV light microscope in which the low-pass filter on the sensitive image sensor was removed. In this photographic system, UV images can be observed on the LCD finder and we can adjust the focus in real time. Moreover, we can increase the depth of focus by combining the stack of through-focus images. Subsequently, images will be analyzed to locate UV-absorbing compounds in the tunic.

MATERIALS AND METHODS

Animals

Diplosoma virens colonies were collected by a snorkel diver at about 0.5 m deep in the vicinity of the Ginowan Port Marina, Okinawa, Japan. *Diplosoma simile* colonies were collected at about 0.3 m deep on the reef flat off Odo Beach, Okinawa, Japan. The colonies were detached from rocks or dead coral rubble and brought to the laboratory. To obtain the absorption spectra of the tunic, live colonies were horizontally sliced with a razor blade to 0.5-1 mm thick slices, and slices from the colony surface were used for the spectrometry. For UV microscopic observations, cross-sections of 0.5 mm thickness of live colonies were prepared by hand using a razor blade and mounted on a glass slide with seawater. A colony of *D. virens* was cut into pieces and fixed in 2.5% glutaraldehyde, 0.1 M cacodylate, and 0.45 M sucrose for histological and ultrastructural observation.

Spectrometry

The tunic sheets from the colony surface were briefly soaked in seawater to remove *Prochloron* cells from the specimens. The tunic sheets were almost transparent, while the surface tunic contained zooid oral features. The specimens were directly attached to a quartz cuvette, and the absorption spectra in the range of 250-800 nm were measured using the spectrophotometer Genesis 10S UV-Vis (Thermo Scientific, Wisconsin, USA).

UV microscopy

A mercury lamp house U-ULH (Olympus, Tokyo Japan) was installed on the trans-illumination aperture of a microscope BX50 (Olympus) instead of a halogen lamp house**.** To transmit UV light and cut visible light, UV band-pass filters U340 (Hoya, Saitama Japan) with or without CT 330/20 (Chroma, Vermont, USA) was mounted on the field lens (Fig. 1). The trans-illumination filtered with U340 contained a relatively wide range of UV light, as more than 50% of transmittance ranged from 288 nm to 366 nm (Fig. 1). On the other hand, more than 50% of transmittance for CT330/20 ranged from 320 nm to 338 nm within UV light (< 400 nm), while this filter also transmits visible light depending on the wavelengths (Fig. 1). Accordingly, the combination of U340 and CT330/20 resulted in narrower bands of UV light (320-338 nm) and extensive light reduction. The condenser was removed so it would not absorb UV light. The UV light transmitted through the specimens was imaged through objective lenses (UplanApoFLx4, UplanApoFLx20, UPlanFLx40: Olympus) and an imaging lens (U-TLU: Olympus). The image was captured using a digital camera (Lummix DMC-G5: Panasonic, Tokyo Japan) in which the low-pass filter cutting UV light on the image senor was removed. A stack of through-focus images was captured manually, and thus the vertical distance between the images was not constant. Ten to 30 serial images were selected from the stack and combined to increase the depth of field using the post-processing image software Helicon Focus Pro 6.4.3 (Helicon Soft, Ltd., Kharkov, Ukraine). Each combined images covered approximately 50-100 μm in depth in the observation with the x40 objective lens.

Notably, the light intensity and sensitivity of the image sensor were different among the

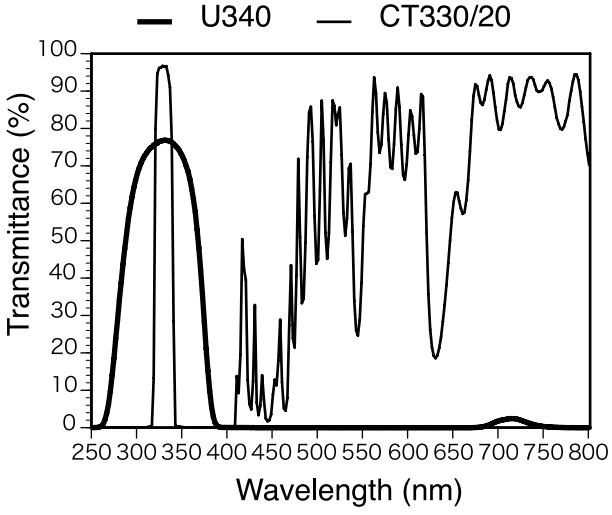

Fig. 1. Transmittance (%) of 250-800 nm light for the band-pass filters U340 (thick line) and CT330/20 (thin line) measured with a spectrophotometer Genesis 10S UV-Vis.

observations using different ranges of wavelengths, and the exposure times for microphotography differed among the combinations of optical filters. Therefore, it is difficult to compare the images obtained under different conditions, but the relative brightness among the tissues and background within each image can be compared.

Electron microscopy

The glutaraldehyde-fixed colony pieces were rinsed with 0.1 M cacodylate and 0.45 M sucrose and post-fixed in 1% osmium tetroxide and 0.1 M cacodylate for 1.5 h. The specimens were then dehydrated with ethanol, cleared with *n*-butyl glycidyl ether, and embedded in epoxy resin. Thick sections (ca. 0.5 μm thick) were stained with toluidine blue for light microscopic observation. Thin sections (ca. 0.1 μm thick) were stained with uranyl acetate and lead citrate and examined using a transmission electron microscope (JEM-1011; JEOL).

RESULTS

The absorption spectra of all the tunic sheets prominently showed a specific UV-R absorption (Fig. 2). Absorption peaks were 325-329 nm in the *Diplosoma virens* colonies and 323-325 nm in the *D. simile* colonies. The absorbance was variable among the specimens.

In the cross-sections, the colonies showed a three-layer structure: surface tunic containing the oral part of the zooids, middle layer comprised of zooids and cloacal cavities, and basal tunic occasionally containing embryos (Fig. 3). The light-blue cells scattered in the tunic were pigmentary tunic cells (or nephrocytes) that reflected the incident light and formed the bluish colony color (rectangle in Fig. 3A). *Prochloron* cells were green spheres, exclusively distributed in the cloacal cavities and never in the tunic. These basic structures were the same in *D. virens* and *D. simile*, as well as other photosymbiotic *Diplosoma* species. The UV images (Fig. 1B, D) showed that the middle layer absorbed UV-R well. While the cloacal cavity filled with *Prochloron* cells strongly absorbed UV-R, the zooid tissues also absorbed UV-R to some extent. Although pigmentary cells of the tunic strongly absorb UV-R (rectangle in Fig. 3B), the surface of and basal tunics of both ascidians did not absorb UV-R wavelengths homogeneously, a situation that may be correlated with the presence of mesh-like structures in its matrix. Additionally, the embryo brooded in the basal tunic was found to absorb UV-R (Fig. 3D), and UV-R absorption around the embryo was almost the same as those in other parts of the tunic.

Figure 4A shows a histological cross-section of the surface tunic and upper-half of the middle layer of *D. virens.* A major part of the surface tunic was occupied by tunic bladder cells that were aggregately distributed, forming an approximately 200 μm thick layer (Fig. 4A). Each bladder cell had a large vacuole. Tunic cells other than tunic bladder cells were also distributed in the matrix. In the middle layer, *Prochloron* cells up to 20 μm in diameter were found in the cloacal cavity. In the UV images at a higher magnification (Fig. 4B-E), the

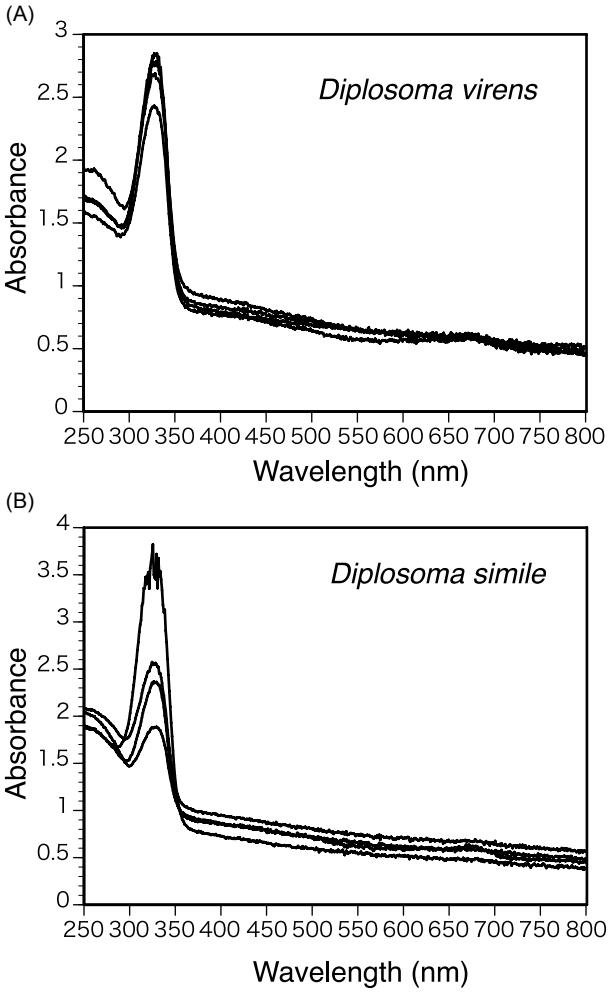

Fig. 2. Absorption spectra of the surface tunic of *Diplosoma virens* (A) and *D. simile* (B). Spectra from four specimens are shown for each species. A prominent absorption peak is present at approximately 325 nm in each spectrum.

periphery of each tunic bladder cell was visualized, indicating absorption of UV-R there. In contrast, there was no UV-R absorption in the vacuolar content of tunic bladder cells. Cell-like objects were also found in the UV images (arrows in Fig. 4B-E). Additionally, UV-R was strongly absorbed by *Prochloron* cells accidentally attached to the tunic surface (arrowhead in Fig. 4C). The image with the light filtered through the combination of U340 and CT330/20 was similar to the image filtered through the U340 filter alone (Fig. 4D, E).

Various types of tunic cells are distributed in the tunic (Fig. 5). In tunic bladder cells, the bulk of the cytoplasm was occupied by a large vacuole, and a thin layer of cytoplasmic matrix enveloped the vacuole (Fig. 5A). The nucleus of the tunic bladder cells was located at one side of the cell periphery, and some cytoplasmic components were found around the nucleus. Certain tunic cells are elongated in shape and are referred to as tunic net cells (Fig. 5B). Additionally, there are other cells that have vacuoles containing electron-dense materials or clear vesicles (Fig. 5C-E).

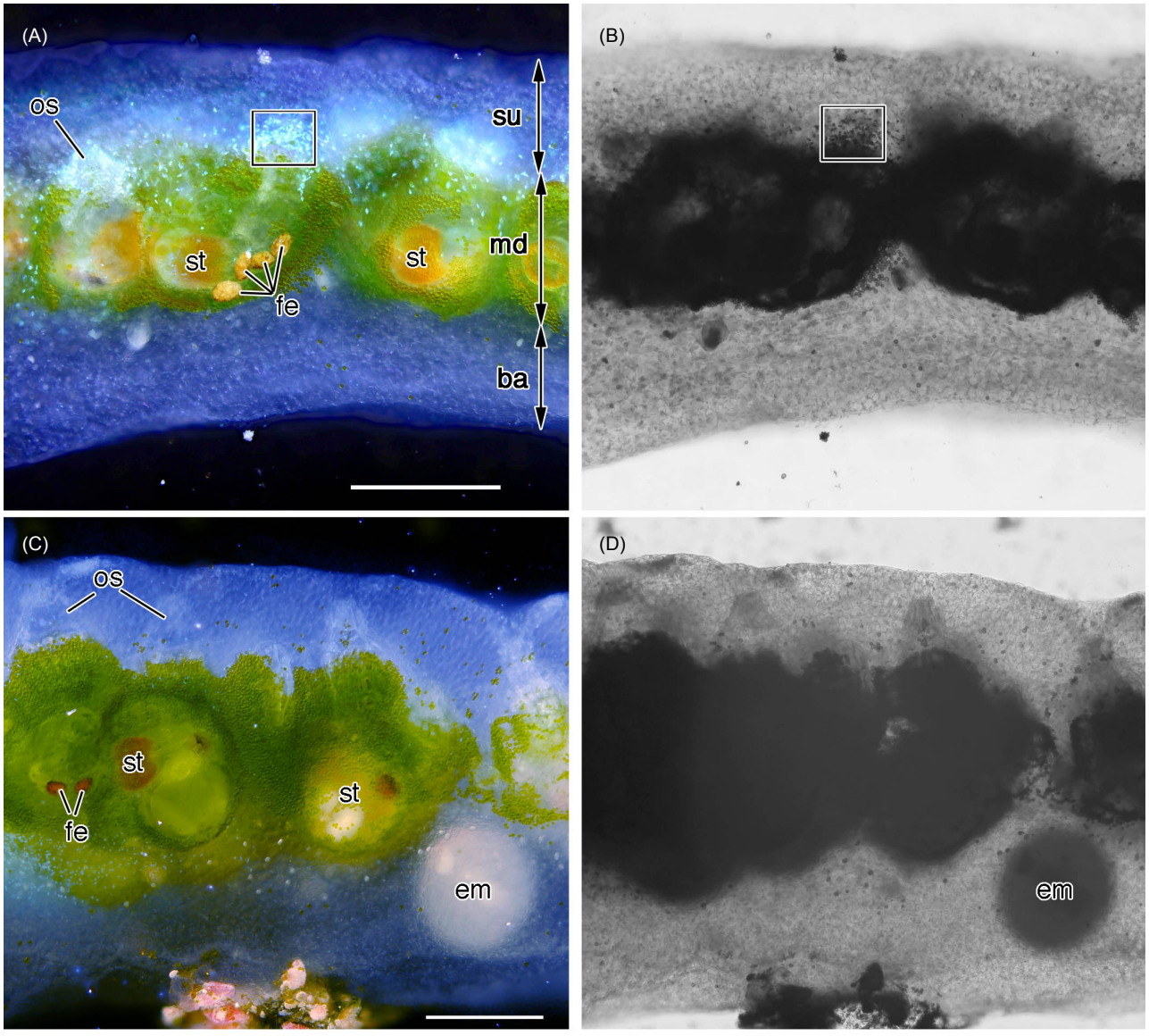

Fig. 3. Pair images of cross-sections of *Diplosoma virens* (A, B) and *D. simile* (C, D) observed with an oblique epi-illumination of white-LED (A, C) and a transmission light filtered with U340 (B, D). The colonies have a three-layer structure: surface tunic (su), middle layer (md), and basal tunic (ba). Rectangles in A and B indicate an aggregate of pigmentary tunic cells. Green areas are cloacal cavities filled with *Prochloron* cells. em, embryo; fe, feces in rectum; os, oral siphon; st, stomach. Scale bars = 0.5 mm.

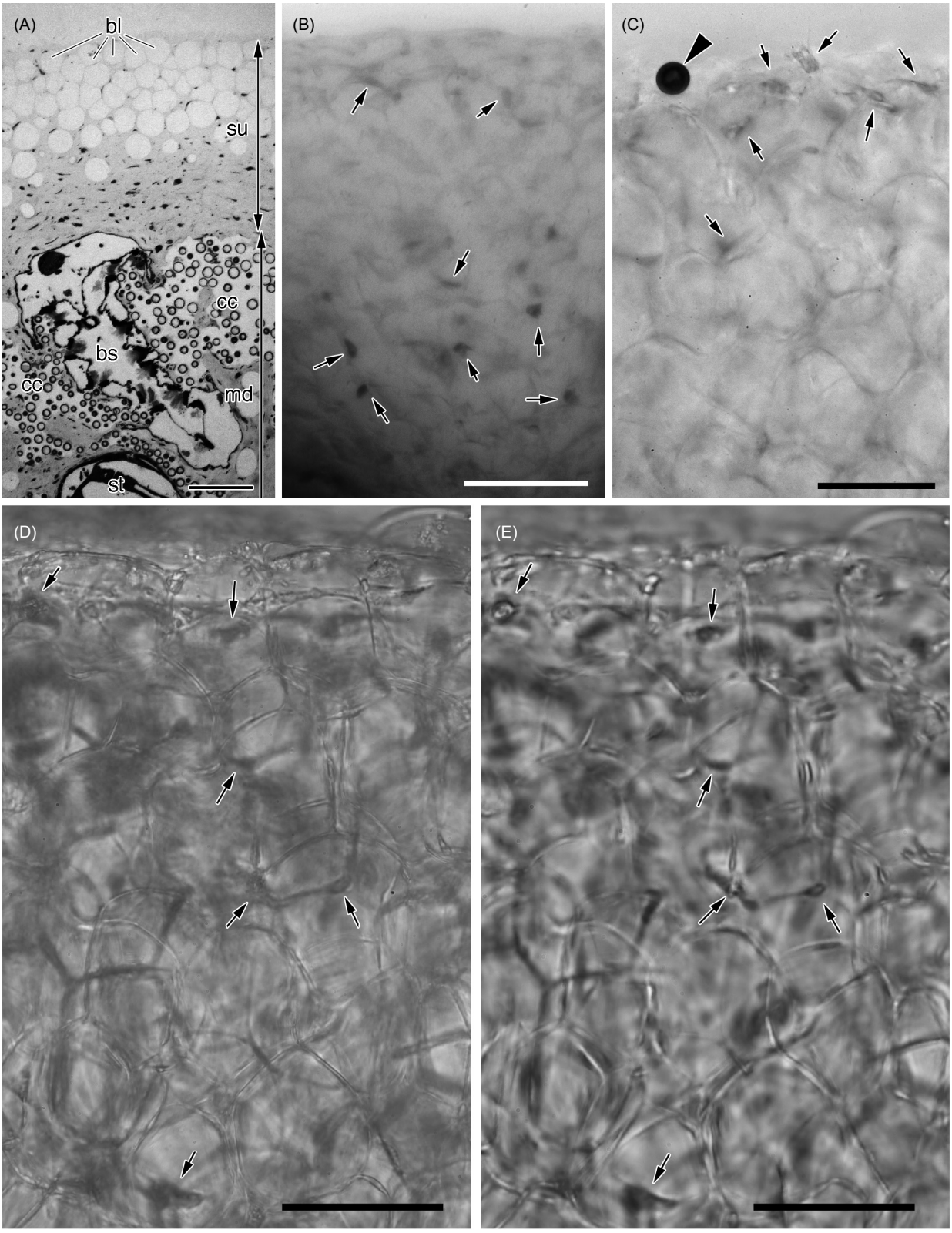

Fig. 4. A, Histological cross-section of the *Diplosoma virens* colony showing surface tunic (su) and middle layer (md). B and C, UV images of the surface tunic of *D. virens* (band-pass filter: U340). D and E, a pair of UV images of the surface tunic of *D. simile* (band-pass filter: U340 for D, U340 + CT330/20 for E). Arrows indicate some cell-like objects absorbing UV-R. Arrowhead in C indicates *Prochloron* cells. bl, tunic bladder cell; bs, branchial sac; cc, cloacal cavity; st, stomach. Scale bars: 100 μm in A, 50 μm in B-E.

DISCUSSION

The UV images in the present study showed that UV-R was absorbed by the cell peripheries of tunic bladder cells, pigmentary tunic cells, and cell-like objects in the tunic of *Diplosoma simile* and *D. virens*, whereas it penetrated tunic matrix and vacuolar content of tunic bladder cells. Moreover, UV-R was well absorbed by *Prochloron* cells distributed in the common cloacal cavity. Ascidian zooids and embryos embedded in the tunic also absorbed UV-R. Absorption of UV-R in the tunic slices is consistent with the results of a previous study (Hirose et al. 2004) and indicates the presence of UV-absorbing compounds in the tunic. The variation in absorbance among specimens is probably due to the differences in tunic sheet thicknesses and amount of UV-absorbing compounds. We did not find a remarkable difference in the UV images between the two species, probably because of their similar habitat. The specimens of the two species were collected in shallow coral reefs at similar depths in the present study, and both species were sometimes recorded at the same sites. Because four MAAs - mycosporine-glycine, palythine, porphyra-334, and shinorine - were isolated from *D. virens* (Hirose et al. 2004), MAAs are potent candidates for the UV-absorbing compounds visualized in the UV images.

In the UV microscopy observations of the colony cross-section, the strongest UV-R absorption was found in the middle layer, probably because *Prochloron* cells contain MAAs (*e.g.*, Dionisio-Sese et al. 1997; Hirose et al. 2004). The UV images at a low magnification proved that mesh-like structures as well as pigmentary tunic cells absorbed UV-R in the surface and basal tunic

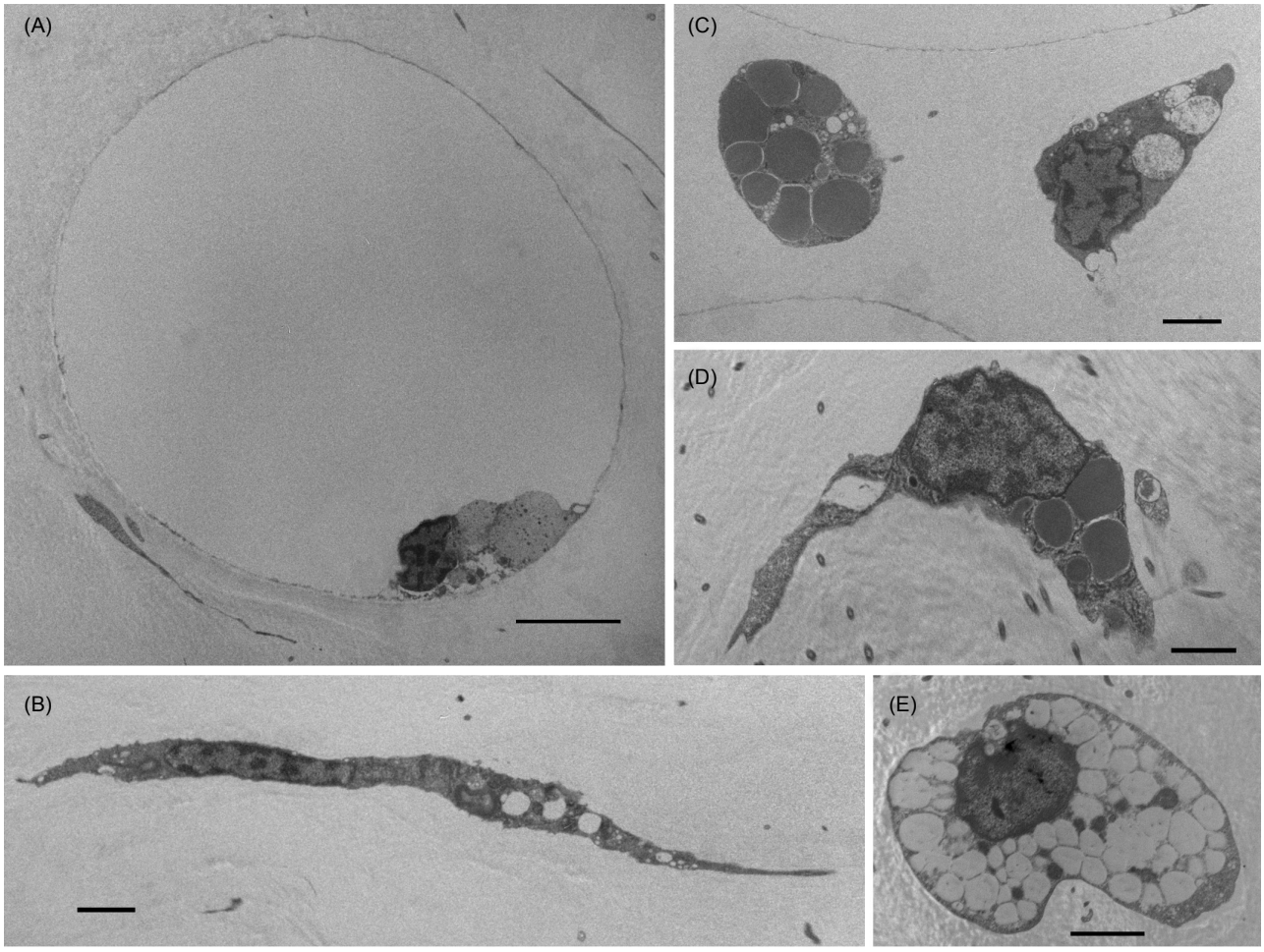

Fig. 5. Tunic cells in the surface tunic of *Diplosoma virens*. A, Bladder tunic cells. B, Tunic net cells (= myocyte *sensu* Mackie and Singla, 1987). C-E, Other types of tunic cells with vacuoles containing electron-dense materials or clear vesicles. Scale bars: 5 μm in A, 2 μm in B-E.

(Fig. 3). Notably, the tunic around the embryo is not specialized to protect the embryo from UV, as UV-R absorption appears the same between the tunic around the embryo and other parts of the tunic (Fig. 3D). Embryos likely receive sufficient protection from UV-R in this condition, as didemnid embryos always occur in the basal tunic where UV-R has already been absorbed in the surface tunic and the middle layer. Moreover, ascidian embryos and accessory cells (test cells and follicle cells) contain MAAs (Epel et al. 1999). In some other invertebrates, MAA concentrations in ovaries and eggs are reported to be higher than other tissues (*e.g.*, Karentz et al. 1992, 1997; Krupp and Blanck 1995; Adams et al. 2001; Michalek-Wagner 2001), probably because the eggs, embryos, and larvae are exposed to higher doses of UV-R than their benthic adults.

Observations at a higher magnification confirmed strong UV-R absorption by *Prochloron* cells. The UV images also showed that the mesh-like structures found at a lower magnification corresponded to UV-R absorption at the cell peripheries of the tunic bladder cells that aggregate and form a layer in the tunic. Some cell-like objects also absorb UV-R. The cell-like objects as well as the cell peripheries of the tunic bladder cells are recognized in the UV images with the trans-illumination filtered by U340 alone and by the combination of U340 and CT330/20. Because the light through the U340 + CT330/20 filters was a narrower band of UV light (320-338 nm), the UV-R absorption peak in the absorption spectra of the tunic (Fig. 2) is consistent with the objects absorbing UV-R in the tunic in figure 4E, *i.e.*, the cell peripheries of the bladder cells and cell-like objects.

There are several types of tunic cells in the *Diplosoma virens*. Bladder tunic cells occupy the largest area in the tunic, aggregating to form a layer, and this cell type is characterized by a large vacuole containing acid fluid probably for chemical defense against predators (Hirose 2001). Considering the UV images, vacuolar content did not absorb UV-R, and the UV-absorbing compounds - such as MAAs - are probably contained in the cytoplasmic matrix. For instance, MAAs are water soluble and can be dissolved in the aqueous phase in the cytoplasm. In tunic bladder cells, while the cytoplasmic matrix between the cell membrane and the vacuolar membrane forms a very thin layer, the other part of the matrix is located at one side of the cell periphery with the nucleus (Fig. 5A). The cytoplasmic matrix around the nucleus is also a candidate for the cell-like objects in the UV images. Tunic net cells form a cellular network in the tunic, connecting their pseudopodia with one another (Hirose 2001). Mackie and Singla (1987) described this type of tunic cells as myocytes in *Diplosoma listerianum* and *D. macdonaldi*, indicating that these tunic cells propagate impulses and contractions in the tunic. Other types of tunic cells are often amoeboid in shape. We cannot specify which of these tunic cells absorb UV-R, as several types of cells potentially contain UV-absorbing compounds in the cytoplasm and absorb UV-R in the tunic.

The cytoplasmic distribution of the UV-absorbing compounds appears consistent with protection of DNA in the nucleus. UV-R damages DNA structures, such as the production of cyclobutane pyrimidine dimer (Setlow and Setlow 1962), and field doses of UV-R damage DNA in marine invertebrates living in the intertidal zone and shallow depths (Lamare et al. 2007; Cubillos et al. 2015). Therefore, the UV-absorbing compounds in the tunic cells form a layer that absorbs UV-R in the tunic to protect cyanobacterial symbionts in the common cloacal cavity from UV-R, and they also protect the tunic cells themselves.

In the UV micrographs of Maruyama et al. (2003), objects that absorbed UV-R were round or cup-shaped and 30-43 μm in diameter, and the authors concluded that the objects absorbing UV-R corresponded to tunic bladder cells. Considering the cup shape of the objects absorbing UV-R, they assumed that “the UV-absorbing substance is contained in the cytoplasm of the bladder cell, and that the large vacuole lacks this substance” (Maruyama et al. 2003). These observations are consistent with those of the present study; however, the UV images were different between the two studies, as the tunic bladder cells were round or cup-shaped in the film-based images (Maruyama et al. 2003), while the cell periphery was visualized in the digital images (present study). Although we cannot specify the practical causes, these differences are probably due to the differences in the methodology used to obtain the UV images, such as the characteristics of photoreceptors (silver salt film and digital sensor) and the combinations of the band-pass filters. Additionally, Maruyama et al. (2003) did not mention cell-like objects (arrows in Fig. 4B-E), probably because they used lower magnification than the present study.

CONCLUSIONS

UV microscopy visualized the histological distribution of UV-R absorption in the tunic. Based on the images, the UV-absorbing compounds are contained in the cytoplasmic matrix at the cell periphery, around the nucleus in tunic bladder cells, and possibly in the cytoplasm of certain other cells. On the other hand, the tunic matrix and vacuolar content of tunic bladder cells appeared to not absorb UV-R. MAAs are potent candidates for UV-absorbing compounds in the present animals because several MAAs were isolated from *Diplosoma virens* (Hirose et al. 2004). Except for some cnidarians that possess genes for the biosynthesis of MAAs (Shinzato et al. 2011), metazoans are generally unable to synthesize MAAs because they lack the synthetic pathway. In photosymbiotic ascidians, cyanobacterial symbionts are the most likely candidates as the source of the MAAs in the tunic because specific absorption of UV-R has never been reported in tunics of non-photosymbiotic ascidians. In *D. virens*, the composition of MAAs was very similar but not identical between isolated *Prochloron* cells and the colony residue from which the *Prochloron* cells were isolated (Hirose et al. 2004). This may suggest that there is another source of MAAs, such as diet, but the cyanobacterial symbionts are likely the major source of MAAs. Therefore, it would be necessary for the tunic cells absorbing UV-R to obtain MAAs synthesized by *Prochloron* cells. However, the tunic cells are separated from *Prochloron* cells in the cloacal cavity by the dense layer of tunic cuticle, and the mechanism for the translocation of MAAs remains unknown.

List of abbreviations

MAAs, mycosporine-like amino acids.
PAR, photosynthetically active radiation.
UV, ultraviolet.
UV-R, ultraviolet radiation.

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Authors' contributions: NS performed UV microscopy. EH prepared the specimens and performed spectrometry and electron microscopy. Both authors participated in revising the manuscript and approved the final manuscript.

Competing interests: NS and EH declare that they have no conflict of interest.

Availability of data and materials: The datasets supporting the conclusions of this article are included within the article.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

REFERENCES

Adams NL, Shick JM, Dunlap WC. 2001. Selective accumulation of mycosporine-like amino acids in ovaries of the green sea urchin *Strongylocentrotus droebachiensis* is not affected by ultraviolet radiation. Mar Biol **138:**281-294. doi:10.1007/s002270000464.

Balskus EP, Walsh CT. 2010. The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. Science **329:**1653-1656. doi:10.1126/science.1193637.

Bingham BL, Reizel AM. 2000. Solar damage of the solitary ascidian, *Corella inflata*. J Mar Biol Assoc UK **80:**515-521. doi:10.1017/S0025315400002216.

Bingham BL, Reyns NB. 1999. Ultraviolet radiation and distribution of the solitary ascidian *Corella inflata* (Huntsman). Biol Bull **196:**94-104. doi:10.2307/1543171.

Carreto JI, Carignan MO. 2011. Mycosporine-like amino acids: Relevant secondary metabolites. Chemical and ecological aspects. Mar Drugs **9:**387-446. doi:10.3390/md9030387.

Cubillos VM, Burritt DJ, Lamare MD, Peake BM. 2015. The relationship between UV-irradiance, photoprotective compounds and DNA damage in two intertidal invertebrates with contrasting mobility characteristics. J Photochem Photobiol B **149:**280-288. doi:10.1016/j.jphotobiol.2015.06.001.

Dionisio-Sese ML, Ishikura M, Maruyama T, Miyachi S. 1997. UV-absorbing substances in the tunic of a colonial ascidian protect its symbiont, *Prochloron* sp., from damage by UV-B radiation. Mar Biol **128:**455-461. doi:10.1007/s002270050112.

Dionisio-Sese M, Maruyama T, Miyachi S. 2001. Photosynthesis of *Prochloron* as affected by environmental factors. Mar Biotechnol **3:**74-79. doi:10.1007/s101260000062.

Donia MS, Fricke WF, Partensky F, Cox J, Elshahawi S, White JR, Phillippy AM, Schatz MC, Piel J, Haygood MG, Ravel J, Schmidt EW. 2011. Complex microbiome underlying secondary and primary metabolism in the tunicate-*Prochloron* symbiosis. Proc Natl Acad Sci USA **108:**E1423-E1432. doi:10.1073/pnas.1111712108.

Dunlap WC, Yamamoto Y. 1995. Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine. Comp Biochem Physiol **112B:**105-114. doi:10.1016/0305-0491(95)00086-N.

Epel D, Hemela K, Shick M, Patton C. 1999. Development in the floating world: Defenses of eggs and embryos

against damage from UV radiation. Am Zool **39:**271-278. doi:10.1093/icb/39.2.271.

Hernández Moresino RD, Helbling EW. 2010. Combined effects of UVR and temperature on the survival of crab larvae (Zoea I) from Patagonia: The role of UV-absorbing compounds. Mar Drugs **8:**1681-1698. doi:10.3390/md8051681.

Hirabayashi S, Kasai F, Watanabe MM, Hirose E. 2006. Contents of ultraviolet-absorbing substances in two color morphs of the photosymbiotic ascidian *Didemnum molle*. Hydrobiologia **571:**419-422. doi:10.1007/s10750-006-0244-7.

Hirose E. 1999. Pigmentation and acid storage in the tunic: protective functions of the tunic cells in the tropical ascidian *Phallusia nigra*. Invertebr Biol **118:**414-422. doi:10.2307/3227010.

Hirose E. 2009. Ascidian tunic cells: morphology and functional diversity of free cells outside the epidermis. Invertebr Biol **128:**83-96. doi:10.1111/j.1744-7410.2008.00153.x.

Hirose E. 2001. Acid containers and cellular networks in the ascidian tunic with special remarks on ascidian phylogeny. Zool Sci **18:**723-731. doi:10.2108/zsj.18.723.

Hirose E. 2015. Ascidian photosymbiosis: Diversity of cyanobacterial transmission during embryogenesis. Genesis **53:**121-131. doi:10.1002/dvg.22778.

Hirose E, Hirabayashi S, Hori K, Kasai F, Watanabe MM. 2006. UV protection in the photosymbiotic ascidian *Didemnum molle* inhabiting different depths. Zool Sci **23:**57-63. doi:10.2108/zsj.23.57.

Hirose E, Maruyama T. 2004. What are the benefits in the ascidian-*Prochloron* symbiosis? Endocytobiosis Cell Res **15:**51-62.

Hirose E, Neilan BA, Schmidt EW, Murakami A. 2009. Enigmatic life and evolution of *Prochloron* and related cyanobacteria inhabiting colonial ascidians. *In*: Gault PM, Marler HJ (eds) Handbook on Cyanobacteria (eds.), Nova Sci Publ, Inc., New York, pp. 161-189.

Hirose E, Ohtsuka K, Ishikura M, Maruyama T. 2004. Ultraviolet absorption in ascidian tunic and ascidian-*Prochloron* symbiosis. J Mar Biol Assoc UK **84:**789-794. doi:10.1017/S0025315404009956h.

Hirose E, Sakai D, Shibata T, Nishii J, Mayama H, Miyauchi A, Nishikawa J. 2015. Does the tunic nipple array serve to camouflage diurnal salps? J Mar Biol Assoc UK **95:**1025-1031. doi:10.1017/s0025315415000119.

Kakiuchida H, Sakai D, Nishikawa J, Hirose E. 2017. Measurement of refractive indices of tunicates' tunics: light reflection of the transparent integuments in an ascidian *Rhopalaea* sp. and a salp *Thetys vagina*. Zool Lett **3:**7. doi:10.1186/s40851-017-0067-6.

Karentz D. 2001. Chemical defenses of marine organisms against solar radiation exposure: UV-absorbing mycosporine-like amino acids and scytonemin. *In*: McClintok JB, Baker BJ (eds) Marine Chemical Ecology. CRC Press, Boca Raton, pp. 481-520.

Karentz D, Dunlap WC, Bosch I. 1992. Distribution of UV-absorbing compounds in the Antarctic limpet, *Nacella concinna*. Antarct J US **27:**121-122.

Karentz D, Dunlap WC, Bosch I. 1997. Temporal and spatial occurrence of UV-absorbing mycosporine-like amino acids in tissues of the antarctic sea urchin *Sterechinus neumayeri* during springtime ozone-depletion. Mar Biol **129:**343-353. doi:10.1007/s002270050174.

Kott P. 2001. The Australian Ascidiacea, part 4, Aplousobranchia (3), Didemnidae. Mem Queensl Museum **47:**1-408.

Krupp DA, Blanck J. 1995. Preliminary report on the occurrence of mycosporine-like amino acids in the eggs of the Hawaiian scleractinian corals *Montipora verrucosa* and *Fungia scutaria*. *In*: Gulko D, Jokiel PL (eds) Ultraviolet Radiation and Coral Reefs. Sea Grant Technical Report no. 41, University of Hawai'i, Honolulu, pp. 129-34.

Kühl M, Behrendt L, Trampe E, Qvortrup K, Schreiber U, Borisov SM, Klimant I, Larkum AWD. 2012. Microenvironmental ecology of the chlorophyll *b*-containing symbiotic cyanobacterium Prochloron in the didemnid ascidian *Lissoclinum patella*. Front Microbiol **3:**402. doi:10.3389/fmicb.2012.00402.

Lamare MD, Barker MF, Lesser MP. 2007. *In situ* rates of DNA damage and abnormal development in Antarctic and non-Antarctic sea urchin embryos. Aquat Biol **1:**21-32. doi:10.3354/ab00003.

Lesser MP, Stochaj WR. 1990. Photoadaptation and protection against active forms of oxygen in the symbiotic prokaryote *Prochloron* sp. and its ascidian host. Appl Environ Microbiol **56:**1530-1535.

Lister KN, Lamare MD, Burritt DJ. 2010. Sea ice protects the embryos of the Antarctic sea urchin *Sterechinus neumayeri* from oxidative damage due to naturally enhanced levels of UV-B radiation. J Exp Biol **213:**1967-1975. doi:10.1242/jeb.039990.

Mackie GO, Singla CL. 1987. Impulse propagation and contraction in the tunic of a compound ascidian. Biol Bull **173:**188-204.

Maruyama T, Hirose E, Ishikura M. 2003. Ultraviolet-light-absorbing tunic cells in didemnid ascidians hosting a symbiotic photo-oxygenic prokaryote, *Prochloron*. Biol Bull **204:**109-113.

Michalek-Wagner K. 2001. Seasonal and sex-specific variations in levels of photo-protecting mycosporine-like amino acids (MAAs) in soft corals. Mar Biol **139:**651-660. doi:10.1007/s002270100625.

Monniot F, Monniot C. 2001. Ascidians from the tropical western Pacific. Zoosystema **23:**201-383.

Piazena H, Perez Rodrigues E, Häder D-P, Lopez Figueroa F. 2002. Penetration of solar radiation into the water column of the central subtropical Atlantic Ocean - optical properties and possible biological consequences. Deep-Sea Res II **49:**3513-3528. doi:10.1016/S0967-0645(02)00093-0.

Sakai D, Kakiuchida H, Nishikawa J, Hirose E. 2018. Physical properties of the tunic in the pinkish-brown salp *Pegea confoederata* (Tunicata: Thaliacea). Zool Lett **4:**7. doi:10.1186/s40851-018-0091-1.

Setlow RB, Setlow JK. 1962. Evidence that ultraviolet-induced thymine dimers in DNA cause biological damage. Proc Natl Acad Sci USA **48:**1250-1257. doi:10.1073/pnas.48.7.1250.

Schmidt EW. 2015. The secret to a successful relationship: lasting chemistry between ascidians and their symbiotic bacteria. Invertebr Biol **134:**88-102. doi:10.1111/ivb.12071.

Shick JM, Dunlap WC. 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu Rev Physiol **64:**223-262.

Shick JM, Dunlap WC, Pearse JS, Pearse VB. 2002. Mycosporine-like amino acid content in four species of sea anemones in the genus *Anthopleura* reflects phylogenetic but not environmental or symbiotic relationships. Biol Bull

203:315-330.
Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M, Fujiwara M, Koyanagi R, Ikuta T, Fujiyama A, Miller DJ, Satoh N. 2011. Using the *Acropora digitifera* genome to understand coral responses to environmental change. Nature **476:**320-323. doi:10.1038/nature10249.
Tedetti M, Semperv R. 2006. Penetration of Ultraviolet radiation in the marine environment. A review. Photochem Photobiol **82:**389-397. doi:10.1562/2005-11-09-IR-733.