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Exumbrellar Surface of Jellyfish: A Comparative Fine Structure Study with Remarks on Surface Reflectance

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The exumbrellar surfaces of six pelagic cnidarians from three classes were ultra-structurally compared to reveal their structural diversity in relation to their gelatinous, transparent bodies. We examined two hydrozoans (*Diphyes chamissonis* and *Colobonema sericeum*), a cubozoan (*Chironex yamaguchii*), and three scyphozoans (*Atolla vanhöffeni*, *Aurelia coerulea*, and *Mastigias papua*). The exumbrellar surfaces of the mesoglea in *D. chamissonis*, *Ch. yamaguchii*, *Au. coerulea*, and *M. papua* were covered with a simple epidermis; the shapes of the epidermal cells were remarkably different among the species. The epidermal cells of *Ch. yamaguchii* and *M. papua* possessed an array of microvilli on the apical side. The array possibly reduced light reflectance and provided some other surface properties, as seen for the cuticular nipple array in tunicates, considering the length, width, and pitch of the microvilli. The reduction of light reflectance on the array of microvilli was supported by the simulation with rigorous coupled wave analysis (RCWA). Microvilli were sparse and did not form an array in metephyrae of *Au. coerulea*. The mesoglea matrix beneath the basal side of the epidermis was loose in all of the species. The exumbrellar side of the mesoglea was exposed only in the mesopelagic species, *At. vanhöffeni* and *Co. sericeum*, and electron-dense layer(s) covered the surface of the mesoglea. It is uncertain whether the exumbrellar epidermis is absent in these species or the epidermal cells are completely exfoliated during the sampling and handling processes. In the latter case, the electron-dense layer(s) on the mesoglea surface might originally underlie the epidermis.

Key words: Cnidaria, epidermal cell, gelatinous plankton, medusa, mesoglea surface, rigorous coupled wave analysis (RCWA)

INTRODUCTION

Terrestrial animals, especially those with considerable body sizes, usually have sturdy skeletal structures to support their body against gravity. However, some aquatic organisms do not possess such hard tissue in water columns; they are called “gelatinous plankton”, which includes jellyfish, comb jellyfish, and pelagic tunicates. Although sturdy structures are also important to support active movement and physical protection of the body, these solid structures tend to be heavy and opaque. A gelatinous body is probably advantageous for a life of floating that often lacks active motility and strong armor. By modeling energy budgets, Dölger et al. (2019) found that there are two equally successful strategies in planktonic filter feeders: having a small, high-energy (dense) body, or a large, low-energy (gelatinous) body. Since the bodies of gelatinous plankton are too soft to protect against physical attacks from their predators, they usually have a transparent body which helps them avoid being found by the predators. However, a transparent body is not always sufficient to be invisible in the euphotic zone; the contours of the body can often be recognized by the reflection of light on the body surface. Therefore, being able to reduce reflections is a crucial function for gelatinous plankton.

Some salps that are found in the epipelagic layer during the daytime have an array of nipple-like protuberances (nipple array) on their body surfaces (Hirose et al., 1999). To date, these structures have not been found in the salp species that occur in deeper (= darker) layers. Thus, the nipple

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arrays on the body surfaces of epipelagic salps are probably related to the visibility of the animals in the photic layer (Hirose et al., 2015). Nipple arrays are known to reduce light reflection in insects (e.g., Bernhard, 1967; Wilson and Hutley, 1982). Results from our previous studies based on a mathematical simulation also indicated that the nipple array on the body surface reduced light reflection under water (Hirose et al., 2015; Kakiuchida et al., 2017; Sakai et al., 2018, 2019). This raised the question of whether similar structural adaptations can be found on the body surfaces of other taxa in gelatinous plankton as well.

Jellyfish, or medusae, is the name given to the planktonic stage of the species of the phylum Cnidaria, which is an animal phylum characterized by cnidocytes that contain nematocysts. The medusa stage occurs in the life cycle of all cnidarian classes other than Anthozoa and some hydroids, e.g., *Hydra*. Molecular phylogenies supported the monophyly of the jellyfish-containing clade, Medusozoa (e.g., Kayal et al., 2013), while the shape, size, lifetime, and ecology of individuals vary among species. Some are only a maximum of a few millimeters wide, while some grow over 1 m wide. Some are commonly found in shallow water, while others occur below the euphotic zone. One of the most prominent organs of jellyfish is usually an umbrella (or bell) which is probably the most conspicuous target for visual predators. In general, the bulk of the cnidarian body is occupied by the mesoglea, which is the gelatinous extracellular matrix, and the outer and inner surfaces of the mesoglea are sandwiched between the epidermis and gastrodermis. Therefore, the body surface of jellyfish is assumed to be entirely covered with the epidermis. This structure is quite different from that of salps, in which the outermost surface of the body is a cellulosic extracellular matrix covering the epidermis. In this study, we compared the ultrastructure of the exumbrellar (aboral side) surface in six species belonging to three cnidarian classes, including two species from the mesopelagic zone, to reveal the presence or absence of structural diversity in the exumbrellar surface, with remarks on the functional nanostructures on the body surface.

MATERIALS AND METHODS

Animals

An individual of *Colobonema sericeum* Vanhöffen, 1902 (Hydrozoa: Trachymedusae: Rhopalonematidae) with umbrella diameter of approximately 3 cm was collected with an oblique tow of the ORI net at station SR1 (35°02′06″N, 138°40′16″E) in Suruga Bay, Japan, on 10 November, 2018 during the cruise of RV Hokuto, SRM-18-11-1. The maximum depth the net reached was 871 m.

Four individuals of *Diphyes chamissonis* Huxley, 1859 (Hydrozoa: Siphonophorae: Diphyidae) in the polygastric stage were collected with a modified NORPAC-net to reduce the damage to the animals at station JE3 (33°49′40″N, 132°34′40″E) in Seto Inland Sea, Japan, during the cruise of the RV Isana on 13 November, 2019. The maximum depth of the net was 40 m. The length of the solitary (anterior) nectophore was approximately 8 mm.

An individual of *Chironex yamaguchii* Lewis & Bentlage, 2009 (Cubozoa, Chirodropida, Chirodropidae) was collected near the sea surface with a bucket in Yagachi Fishery Port (Yagachijima Is., Okinawa, Japan) on 14 October, 2019. The umbrella diameter was approximately 15 cm.

An individual of *Atolla vanhöffeni* Russell, 1957 (Scyphozoa: Coronate: Atollidae) was collected with the IKMT (Isaccs-Kid Midwater Trawl) at station NPE6 (36°00′17″N, 141°32′33″E) in the North Pacific, on 28 September, 2018 during the cruise of the RV Shinsei Maru, KS-18-12. The maximum depth the net reached was 1151 m. The umbrella diameter was approximately 2.5 cm.

An individual of *Aurelia coerulea* von Lendenfeld, 1884 (Scyphozoa: Semaeostomeae: Ulmaridae) was collected near the sea surface with a bucket in Orido Bay, Shizuoka, Japan (34°59′31″N, 138°30′39″E) on 4 April, 2019. The umbrella diameter was 12.5 cm. Planula larvae of *Au. coerulea* were obtained from a single medusae collected at the same site and were grown to strobilae in the laboratory. Ephyra larvae released from the strobila were grown into young medusae with bell diameters of ca. 4 mm.

An individual of *Mastigias papua* (Lesson, 1830) (Scyphozoa: Rhizostomeae: Mastigiidae) was collected near the sea surface with a hand net off Hamahigajima Is. (Okinawa, Japan) on 28 July, 2019. The umbrella diameter was approximately 3.5 cm. Young medusae of *M. papua* were also obtained from laboratory-cultured polyps of the species. They were reared in the laboratory from planula larvae released by an adult female of the species collected in Minami-Izu, Japan on 31 August, 2017. The metephyra with about 3 mm bell diameter was fixed for microscopic investigation.

Species identification were carried out based on Kramp (1961) and Totton (1965).

Electron microscopy

Colobonema sericeum and *At. vanhöffeni* specimens were fixed in 2.5% glutaraldehyde-seawater (pH 7.4), and the umbrellas were cut into pieces of approximately 5 × 5 mm with scissors in the fixative solution. Specimens of *D. chamissonis* in the polygastric stage were fixed in 2.5% glutaraldehyde–0.45 M sucrose–0.1 M cacodylate (pH 7.4) at room temperature. Exumbrellar tissues of approximately 5 × 5 mm were cut off from the umbrellas of *Ch. yamaguchii* and *M. papua* and fixed in 2.5% glutaraldehyde–0.45 M sucrose–0.1 M cacodylate (pH 7.4). *Aurelia coerulea* were fixed in 2.5% glutaraldehyde–0.45 M sucrose–0.1 M cacodylate (pH 7.4), and approximately 5 × 5 mm pieces of their exumbrellar tissues were cut off with a razor blade in the fixative solution. Young medusae

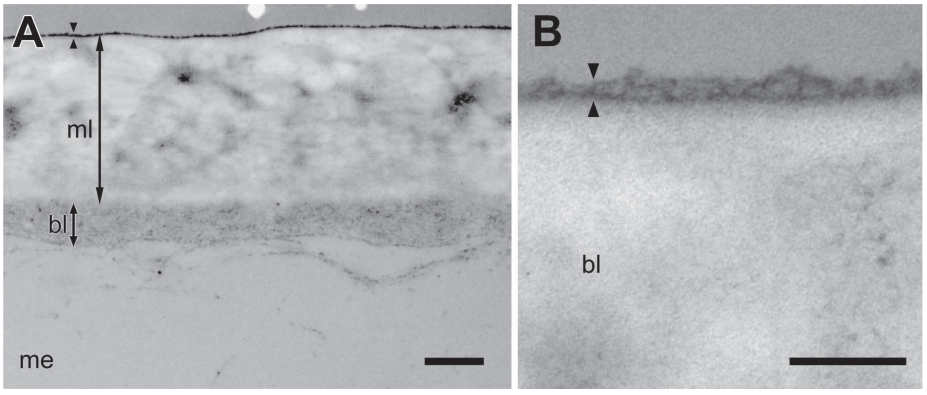

Fig. 1. Exumbrellar surface of *Colobonema sericeum*. **(A)** The exumbrellar surface of the mesoglea is covered with a three-layered sheet, which includes the outer, middle (ml), and basal layers (bl). **(B)** Enlargement of the outer layer overlying the middle layer. Facing arrowheads indicate the outer layer. bl, basal layer; me, mesoglea; ml, middle layer. Scale bars: 0.5 μm for **(A)**, 0.2 μm for **(B)**.

(metephyrae) of *Au. coerulea* and *M. papua* were fixed in 2.5% glutaraldehyde–0.45 M sucrose–0.1 M cacodylate (pH 7.4) at about 23°C. These glutaraldehyde-fixed specimens were stored at 4°C until post fixation.

The specimens were briefly rinsed with 0.1 M cacodylate and 0.45 M sucrose and post-fixed in 1% osmium tetroxide and 0.1 M cacodylate for 1.5–2 h on ice. They were then dehydrated through a graded ethanol series, cleared with *n*-butyl glycidyl ether, and embedded in epoxy resin. The specimens sectioned at a thickness of 0.5–1 μm were stained with 1% toluidine blue and examined under a light microscope (Olympus BX51). Thin sections were stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (TEM; JEOL JEM-1011) at 80 kV.

Simulation of light reflectance on an array of microvilli

To investigate how the array of microvilli affect light reflectance on the exumbrellar surface, we calculated the light reflection and diffraction from the virtual arrays of microvilli in seawater with rigorous coupled wave analysis (RCWA) using DiffractMOD3.2 software (RSoft Design Group, Inc., Ossining, NY). In this simulation, the refractive index of the seawater was defined as 1.343 and that of the exumbrellar epidermis was assumed to be 1.443, considering that the refractive index would not differ much from that of seawater. Both transverse electric wave (TE wave) and transverse magnetic wave (TM wave) were used as the light source. Based on the ultrastructural observations, we assumed that each microvillus consisted of a hemisphere (tip) and a round pillar (body) of 100 nm in diameter. Parameters for the simulation included the following: wavelength of light (λ = 633 nm), incident angle (θ = 75°), height of the microvilli (0, 100, 200, 500, 1000, 2000, and 5000 nm), and pitch (distance between the apices of adjacent microvilli: 100 nm–2 μm).

RESULTS

Ultrastructures of exumbrellar surface

Colobonema sericeum

The exumbrellar surface did not have any cellular components, and a three-layered structure overlaid the umbrella mesoglea (Fig. 1A). The outermost layer was a thin, electron-dense layer 15–30 nm thick. Its surface was irregularly uneven, and the deviation of the surface roughness was approximately 7 nm, as shown in Fig. 1B. The middle layer was a heterogenous matrix, with a thickness of around 1.5 μm. The basal layer was sandwiched between the middle layer and mesoglea. This layer was a fibrous matrix and approximately 0.4 μm in thickness.

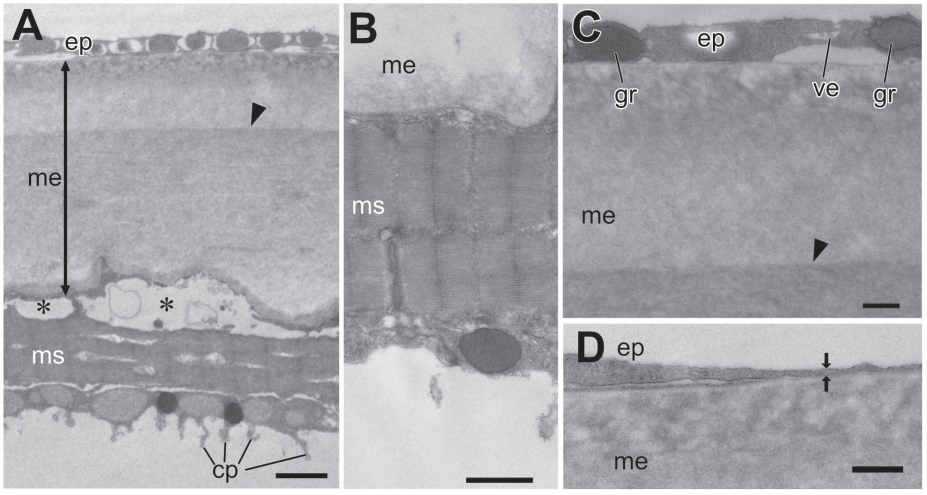

Fig. 2. Bell of *Diphyes chamissonis*. **(A)** Cross section of the bell wall. **(B)** Muscle. **(C)** Epidermis containing granular inclusions. **(D)** Thin layer of epidermis (facing arrows). Arrowheads indicate the border of the different densities in the mesoglea. Asterisks indicate the gaps in the muscular epidermis. cp, cellular process; ep, epidermis; gr, granular inclusions; me, mesoglea; ms, muscle; ve, vesicle. Scale bars: 2 μm for **(A)**, 1 μm for **(B)**, 0.5 μm for **(C**, **D)**.

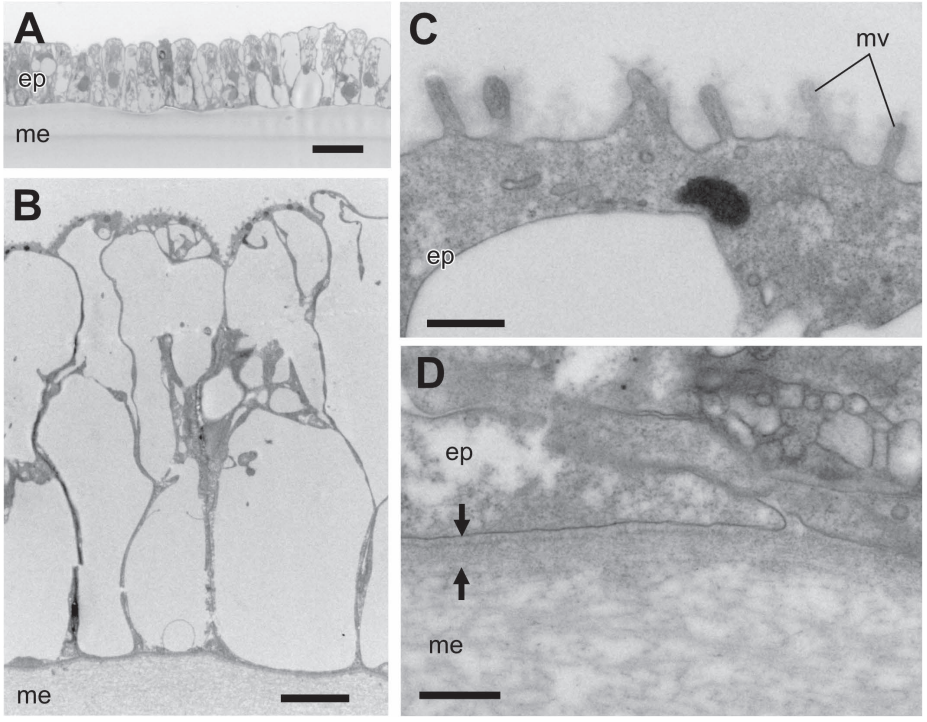

Fig. 3. Exumbrellar surface of *Chironex yamaguchii*. **(A)** Histological section of the epidermis covering the umbrella mesoglea. **(B)** Transmission electron micrographs of the epidermis comprising columnar cells with large vacuoles. **(C)** Apical side of the epidermal cells with short microvilli (mv). **(D)** Basal side of the epidermal cells. Facing arrows indicate the thickened layer of mesoglea. ep, exumbrellar epidermis; me, mesoglea; mv, microvilli. Scale bars: 20 μm for **(A)**, 5 μm for **(B)**, 0.5 μm for **(C**, **D)**.

Diphyes chamissonis

In the cross-sections around the middle point of the bells (nectophore), the bell walls consisted of a very thin outer (= exumbrella) epidermis, mesoglea, and inner (= subumbrella) muscular epidermis (Fig. 2). Although the digestive cavity lined with gastrodermis spreads in typical jellyfish umbrellas, the cavity was not found in this part of the bell in the present species. Whereas the electron density of the mesoglea was high in the exumbrellar surface and gradually decreased toward the subumbrellar side, the density increased abruptly in the middle of the mesogleal layer (arrowheads in Fig. 2A, C). The subumbrellar epidermis extended some cellular processes to the lumen of the bell, and the striated muscle ran circumferentially (Fig. 2A, B). The exumbrellar epidermis was a thin squamous epithelium 0.5–1 μm in thickness and contained granular inclusions and vesicles (Fig. 2C). In a large part of the exumbrella, the surface of the mesoglea was covered with a very thin layer of the epidermis which was less than 0.1 μm in thickness (Fig. 2D). There were no cellular processes or ornaments on the apical surface of the exumbrellar epidermis.

The muscular epidermis often had some gaps in the cytoplasm (asterisks in Fig. 2A), and it was partially detached from the mesoglea. Similarly, the exumbrellar epidermis was often peeled off from the mesoglea. These observations indicated that the specimens were partially damaged during the sampling and handling of the medusae, but subcellular structures appeared to be preserved in the thin sections.

Chironex yamaguchii

The exumbrellar surface of the mesoglea was covered with columnar epidermis approximately 25 μm in height, and these epidermal cells were highly vacuolated (Fig. 3A, B). Microvilli, 0.5 μm in length, emerged on the apical membrane of the epidermal cells, and fuzzy material surrounded the microvilli (Fig. 3C). In the electron micrographs, the average and standard deviation of the width of the microvilli were 98 ± 11 nm (n = 26) and those of the pitch were 365 ± 59 nm (n = 18). The cell membrane was almost flat on the basal side of the cell, and mesoglea matrix formed a thickened layer, approximately 0.2 μm thick, beneath the basal membrane of the epidermis (Fig. 3D).

Aurelia coerulea

Macroscopically, the umbrella of the adult jellyfish had a warty surface on the exumbrellar (aboral) side (Fig. 4A). Each wart was approximately 30–100 μm in height in histological sections, but these values may be underestimated considering the shrinkage of the tissues during the embed-

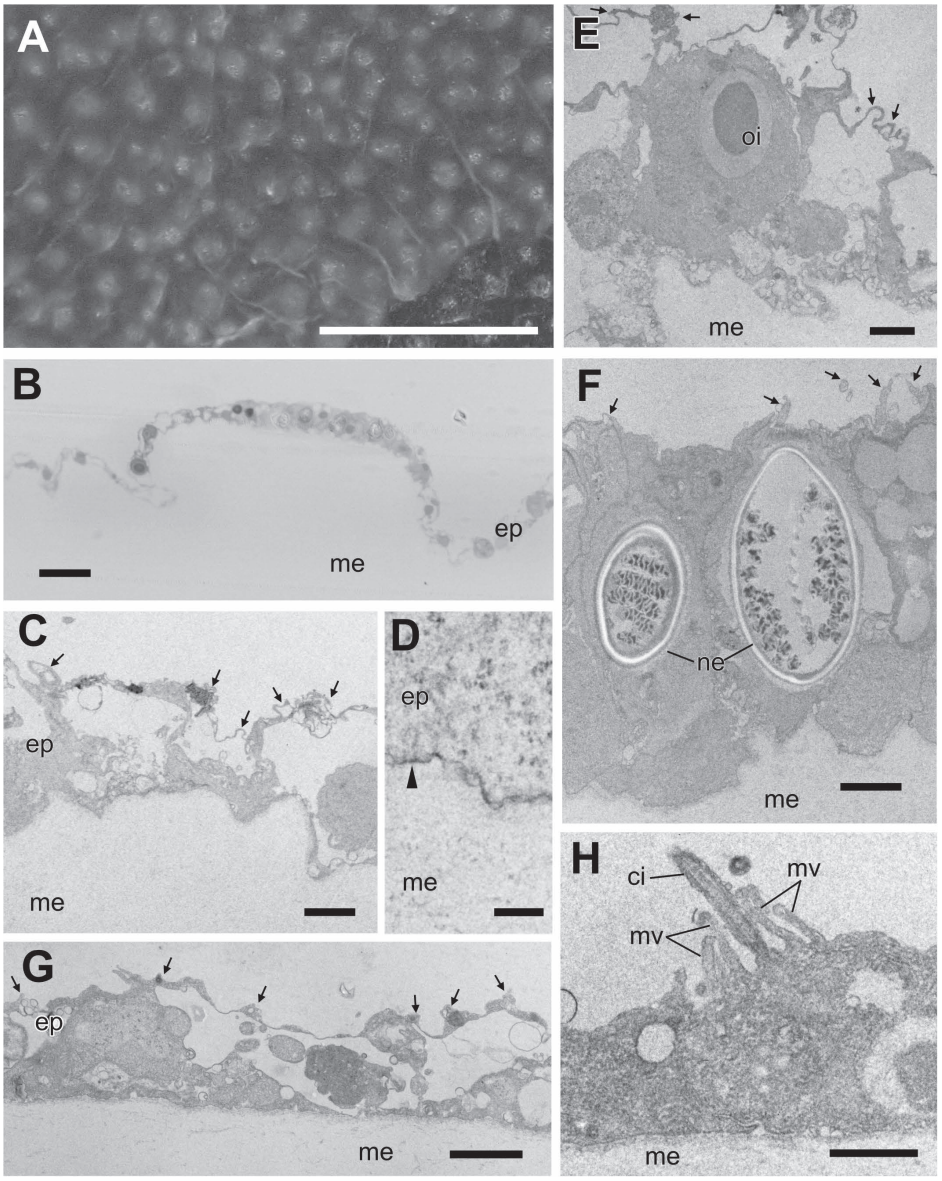

Fig. 4. Exumbrellar surface of *Aurelia coerulea*. **(A)** Exumbrellar (aboral) side of the umbrella showing a warty surface. **(B)** Histological section of the epidermis forming warts on the surface. **(C)** Transmission electron micrographs of the epidermis comprising cuboidal cells with large vacuoles. **(D)** Basal side of the epidermal cell. Arrowhead indicates the basal membrane of the epidermis. **(E)** Epidermal cell containing an oval inclusion. **(F)** Cnidocyte containing nematocysts. **(G)** Epidermis of metephyra. **(H)** A cilium associated with the microvilli in the metephyra epidermis. Arrows indicate cellular bulges and processes. ci, cilium; ep, exumbrellar epidermis; me, mesoglea; mv, microvilli; ne, nematocyst; oi, oval inclusion. Scale bars: 1 mm for **(A)**, 20 μm for **(B)**, 2 μm for **(C, E–G)**, 0.2 μm for **(D)**, 1 μm for **(H)**.

ding process (Fig. 4B). The exumbrellar surface was covered with a simple epidermis that mostly comprised highly vacuolated cuboidal cells 3–10 μm in height (Fig. 4B, C). Cellular bulges and processes in the epidermal cells resulted in an irregularly shaped apical surface of the epidermis. The basal side of the epidermis was irregularly plicate, and mesoglea matrix was not thickened beneath the basal membrane of the epidermis (Fig. 4D). Some epidermal cells contained an oval inclusion consisting of an outer layer and inner core; the electron densities clearly differed between these two parts (Fig. 4E). Cnidocytes that contained nematocysts were occasionally found in the epidermis (Fig. 4F).

As with the adult specimen, a simple epidermis covered the exumbrellar surface in the metephyra specimen (Fig. 4G). A cilium surrounded by microvilli was occasionally found on the apical side of the epidermal cells (Fig. 4H).

Mastigias papua

The exumbrellar surface of the adult jellyfish was covered with a simple epidermis that mostly comprised highly vacuolated cuboidal cells 3–10 μm in height (Fig. 5A). There were numerous microvilli and some cilia on the apical membrane of the epidermal cells (Fig. 5B). In the electron micrographs some long microvilli, over 2 μm in length, were observed; the average and standard deviation of the width were 130 ± 16 nm (n = 36), while those of the pitch were 339 ± 58 nm (n = 28). On the basal side, mesoglea matrix formed a loosely thickened layer of 0.5–1 μm thickness beneath the basal membrane (Fig. 5C).

In the metephyra specimen, a simple epidermis covered the exumbrellar surface. Many of the epidermal cells were vacuolated, and some contained oval inclusions (Fig. 5D). We occasionally found a cilium surrounded by microvilli on the apical side (Fig. 5E) and a nematocyst in the cytoplasm (Fig. 5F).

Atolla vanhöffeni

The exumbrellar surface did not have any cellular components, and an electron-dense layer overlaid the exumbrellar mesoglea (Fig. 6A). The dense layer was 0.3–0.4 μm thick. Its surface was irregularly

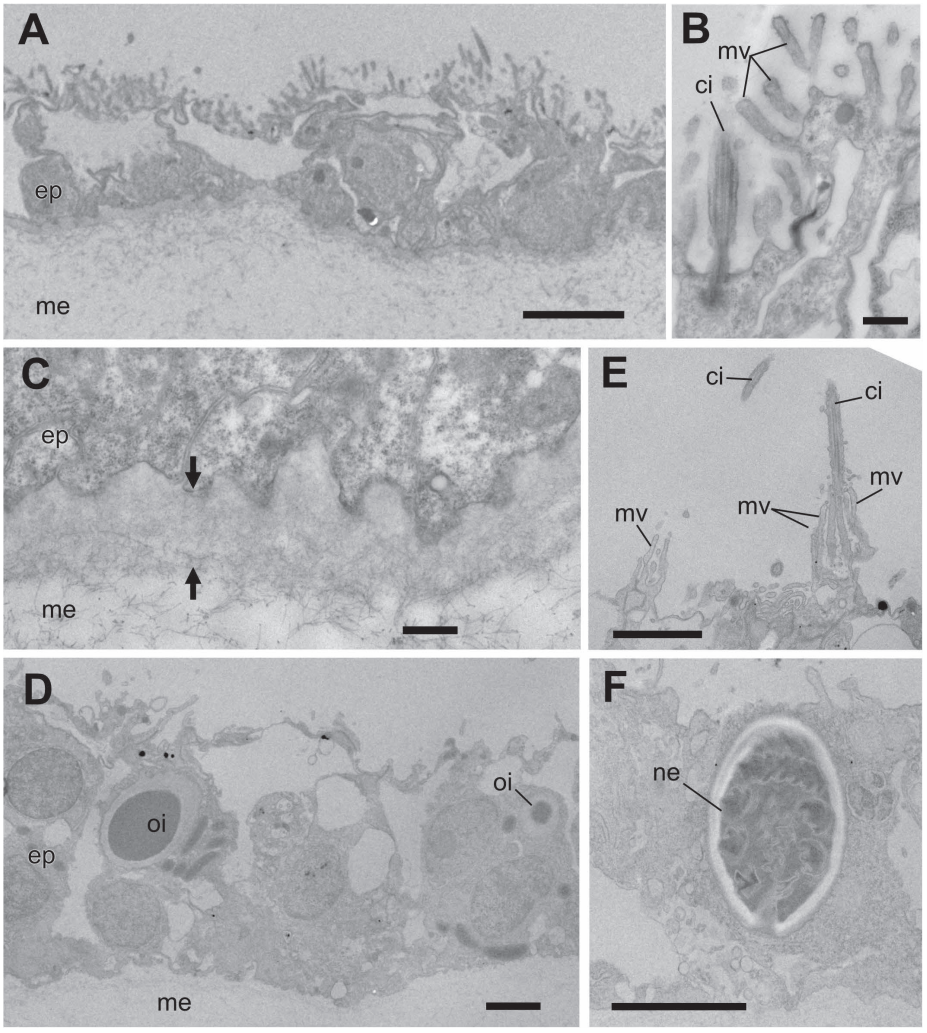

Fig. 5. Exumbrellar surface of *Mastigias papua* (transmission electron micrographs). **(A)** Vacuolated epidermal cells with microvilli on the apical side. **(B)** Microvilli and cilia on the apical side of the epidermal cells. **(C)** Enlargement of the border between the epidermis and mesoglea. Facing arrows indicate the thickened layer of mesoglea. **(D)** Metephyra epidermis containing oval inclusions (oi). **(E)** A cilium associated with microvilli in the metephyra epidermis. **(F)** Nematocyst in the metephyra epidermis. ci, cilium; ep, exumbrellarepidermis; me, mesoglea; mv, microvilli; ne, nematocyst; oi, oval inclusion. Scale bars: 5 μm for **(A)**, 0.5 μm for **(B, C)**, 2 μm for **(D–F)**.

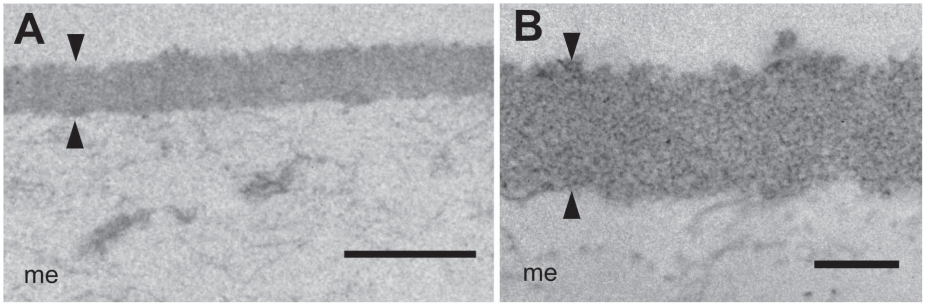

Fig. 6. Exumbrellar surface of *Atolla vanhöffeni.* **(A)** An electron-dense layer covers the exumbrellar surface of the mesoglea (facing arrowheads). **(B)** Enlargement of **(A)**. Scale bars: 1 μm for **(A)**, 0.2 μm for **(B)**.

Table 1. Six species of jellyfish and their exumbrellar structures.

Class	Species	Habitat	Color	Epidermis	Microvilli	Exumbrellar surface of mesoglea
Hydrozoa	*Colobonema sericeum*	Mesopelagic	Semi-transparent	Absent	N/A	Three-layered cuticle
Hydrozoa	*Diphyes chamissonis*	Epipelagic	Transparent	Thin, squamous	Absent	Not specialized
Cubozoa	*Chironex yamaguchii*	Epipelagic	Transparent	Clumnar	Array (height, ca. 0.5 μm; pitch, ca. 365 nm)	Thickened matrix of mesoglea
Scyphozoa	*Atolla vanhöffeni*	Mesopelagic	Transparent with reddish brown tissues	Absent	N/A	Not specialized
Scyphozoa	*Aurelia coerulea*	Epipelagic	Semi-transparent	Cuboidal	Sparse	Loosely thickened matrix of mesoglea
Scyphozoa	*Mastigias papua*	Epipelagic	Transparent with white spots*	Cuboidal	Array (height, over 2 μm; pitch, ca. 340 nm)	Monolayered cuticle

*Brownish due to symbiotic dinoflagellates

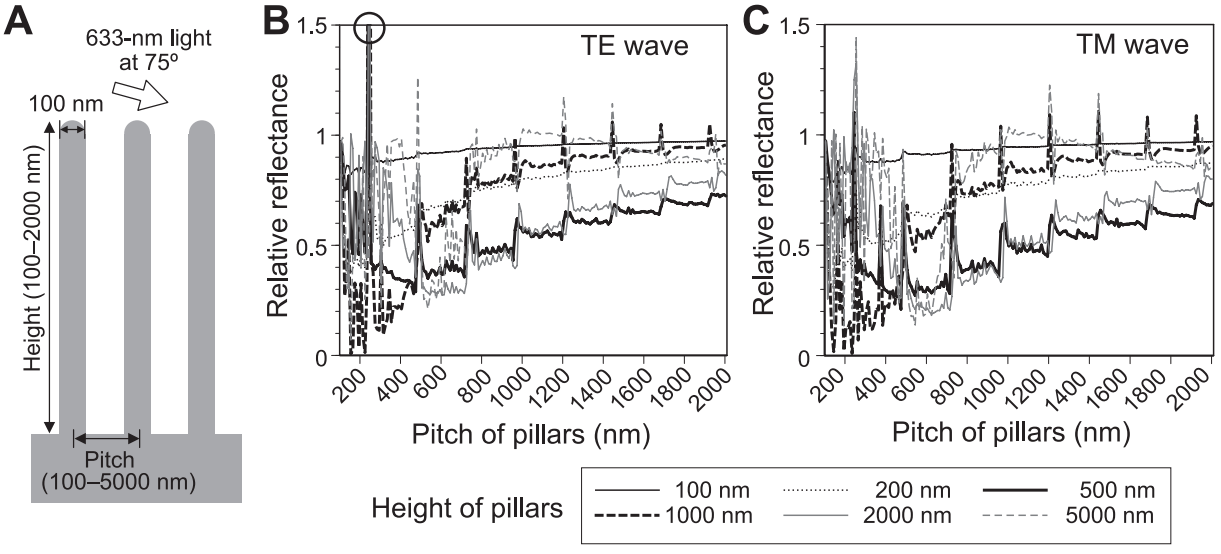

Fig. 7. A model of microvillar array used in the simulation of light reflection and diffraction **(A)**, and the relative reflectance compared with the flat surface for TE wave **(B)** and TM wave **(C)**. The relative reflectance of 500-nm and 1000-nm pillars was beyond the range of the graph at 240-nm pitch (open circle in **(B)**).

uneven, and the height of the profile was 30–40 nm (Fig. 6B).

Table 1 summarizes the ultrastructures of the exumbrellar surface in the six jellyfish species examined here.

Simulation of light reflectance on an array of microvilli

We virtually designed a model of microvillar array (Fig. 7A) and obtained the reflectance of incident light (λ = 633 nm; θ = 75°) with RCWA. This reflectance includes both reflection and diffraction of light on the structures. Figures 7B and 7C respectively show the relative reflectance in the models compared with the reflectance of the flat surface for TE wave and TM wave; relative reflectance less than 1 indicates anti-glare function of the model. The relative reflectance was very different among the models of different pillar height and mostly smaller than 1. However, the relative reflectance drastically fluctuated depending on the pitch of the pillars, and it was occasionally larger than 1. For instance, the relative reflectance of TE wave was 2.12 on the 500-nm pillars and 4.43 on the 1000-nm pillars, when the pitch of the pillar was 240 nm (open circle in Fig. 7B).

In the microscopic observations, the microvilli in *Ch. yamaguchii* were ca. 0.5 μm long at ca. 365 nm pitch and those in *M. papua* were over 2 μm long at ca. 340 nm pitch. When these values were applied to the present simulation, the relative reflectance of 500-nm pillars at 360-nm pitch was 0.35 for TE wave and 0.32 for TM wave and that of 2000-nm pillars at 340-nm pitch was 0.5 for TE wave and 0.7 for TM wave.

DISCUSSION

Cnidarian bodies are usually composed of two layers of epithelial sheets — the epidermis and gastrodermis. The mesoglea, a gelatinous extracellular matrix, is sandwiched between these two epithelia (e.g., Lesh-Laurie and Suchy,

1991; Thomas and Edwards, 1991). Based on this, a simple epidermis should entirely overlie the external surface of umbrella mesoglea on both the exumbrellar and subumbrellar sides, and the internal surface facing the stomach and canal system is lined with gastrodermis. In the present study, the epidermis on the exumbrellar surface was observed in *D. chamissonis, Ch. yamaguchii*, *Au. coerulea*, and *M. papua* which were collected in the epipelagic layer (upper 40 m depth) in coastal areas. In contrast, the exumbrellar surfaces were not covered with a cellular layer in the mesopelagic species, *Co. sericeum* and *At. vanhöffeni*. There could be two possible reasons for the lack of the epidermis. One possibility is that artifacts caused potential damage to the specimens. As the exumbrellar epidermis of the *D. chamissonis* was found to have partly peeled off, it can be speculated that the exumbrellar epidermis might have completely peeled off during the collection of the species owing to the soft and fragile nature of jellyfish tissue. In this case, the surface layers on the mesoglea in these species would correspond to the layer of thickened mesoglea matrix beneath the exumbrellar epidermis that had been artificially lost in the present specimens. The other possibility is the natural absence of an exumbrellar epidermis in these species. *Colobonema sericeum* and *At. vanhöffeni* are known to be distributed in the mesopelagic layer (Roe et al., 1984; Lindsay et al., 2004), where only a small amount of blue light (wavelength 470–480 nm) is attainable (e.g., Denton, 1990). The body color of *At. vanhöffeni* is reddish brown, which is a cryptic color in the mesopelagic layer, but rather conspicuous under the epipelagic light condition. Although the exumbrellar surface of these mesopelagic species is almost flat due to the absence of the epidermis, these jellyfish might not need optically functional structures on the exumbrellar surface under a very low-light condition. The absence of an exumbrellar epidermis may be related to the ambient brightness of the habitats of jellyfish. The observed exumbrellar surfaces were covered with an electron-dense layer in *At. vanhöffeni*, and with a three-layered structure in *Co. sericeum*. In contrast to these surface structures, the thickened layer of mesoglea matrix observed beneath the basal side of the exumbrellar epidermis was a loose fibrous layer in *Ch. yamaguchii* (Fig. 3D) and *M. papua* (Fig. 5C). This structural difference seems to support the possibility that the medusae of *At. vanhöffeni* and/or *Co. sericeum* do not possess an exumbrellar epidermis, and that the exumbrellar surface of the mesoglea is protected by dense or complex layers, at least in full-grown individuals. In the hydrozoan medusa *Polyorchis penicillatus* (Eschscholtz, 1829) and *Aglantha digitale* (Müller OF, 1776), thick fibers in the mesoglea were anchored in the amorphous dense matrix lining the basal side of the exumbrellar epidermis, and the dense matrix formed a laminated structure between the epidermis and mesoglea (Weber and Schmidt, 1985). These observations suggest that the layered structures on the exumbrellar surface were the layer of thickened mesoglea matrix that were exposed following the exfoliation of the epidermis in *At. vanhöffeni* and/or *Co. sericeum* during the growth process. It is necessary to examine young and intact specimens for a better understanding of exumbrellar structures, particularly for species that inhabit the mesopelagic zone.

The cnidarian epidermis includes several types of cells, such as mucus cells and cnidocytes, and the composition and diversity of epidermal cell types differ among tissues and species (Lesh-Laurie and Suchy, 1991; Thomas and Edwards, 1991). The exumbrellar epidermis of medusae is usually composed of thin, flat epidermal cells that are often vacuolated and occasionally contain cnidocytes and mucus cells that often have cilia (e.g., Chapman, 1974; Germer and Hündgen, 1978; Weber and Schmidt, 1985; Hündgen, 1986). In the present study, the epidermal cells were columnar in *Ch. yamaguchii* and cuboidal in *Au. coerulea* and *M. papua*, and they were often highly vacuolated. In *D. chamissonis*, the thin epidermal cells formed extremely thin cellular extensions that covered the mesoglea surface.

In the two scyphozoans, *Au. coerulea* and *M. papua*, we found cnidocytes and cells that possessed cilia in the exumbrellar epidermis. Additionally, we also found conspicuous oval structures that had electron-dense cores in these two species (Figs. 4E and 5D), which are possibly involved in mucus secretion. According to Hündgen (1986), mucus cells are diffusely distributed and generally possess apical cilia. Thus, the ciliated cells in *Au. coerulea* and *M. papua* may have been mucus cells, and the cilia may be potentially responsible for the mucus flow on the exumbrellar surface. The mucus coat is supposed to aid various functions, including food capture and surface cleaning (Tidball, 1986), and Southwards (1955) reported that the ciliary current on the exumbrellar surface carries the particles toward the umbrella edge on the exumbrellar and subumbrellar surface of a scyphozoan, *Aurelia aurita* (Linnaeus, 1758). It is also possible that the ciliary cell is a sensory cell.

Microvilli were found on the exumbrellar epidermis of some cnidarian medusae, and the presence of microvilli seemed to depend on the species. For instance, Weber and Schmidt (1985) described exumbrellar microvilli in *P. penicillatus* but not in *A. digitale,* in their fine structure study on medusae. In the present study, the epidermal cells had an array of microvilli on the apical side of *Ch. yamaguchii* and *M. papua*, whereas microvilli were occasionally found in the metephyrae of *Au. coerulea* but not in *D. chamissonis*. In *Ch. yamaguchii*, each microvillus was approximately 0.5 μm long and did not touch the neighboring microvilli. The microvilli of *M. papua* were much longer than those of *Ch. yamaguchii*, and they appeared tangled with each other. These microvilli might be comparable in function to those of the nipple arrays on the cuticular surfaces of tunicates and parasitic copepods (e.g., Hirose et al., 1990, 1997, 1999; Hirose and Uyeno, 2014), although the length and pitch of the microvilli were greater than those of the nipple arrays. Figure 8 shows simplified schemas of the exumbrellar microvilli and cuticular nipple arrays, although we should consider that the length (height) and densities of the nipples vary among the parts of the body/colony (Hirose et al., 1990; Sakai et al., 2018). The nipple arrays are known to aid various functional properties associated with the body surface: reduction of light reflection (e.g., Bernhard, 1967; Wilson and Hutley, 1982; Hirose et al., 2015), reduction of air bubble adhesion (Hirose et al., 2013) and of debris (Peisker and Gorb, 2010), suppression of cell attachment (Nomura et al., 2005; Ballarin et al., 2015) and of biofouling (Hirose and Sensui, 2019). It is possible that the microvilli provide similar surface properties to the nipple arrays. Among the four epi-

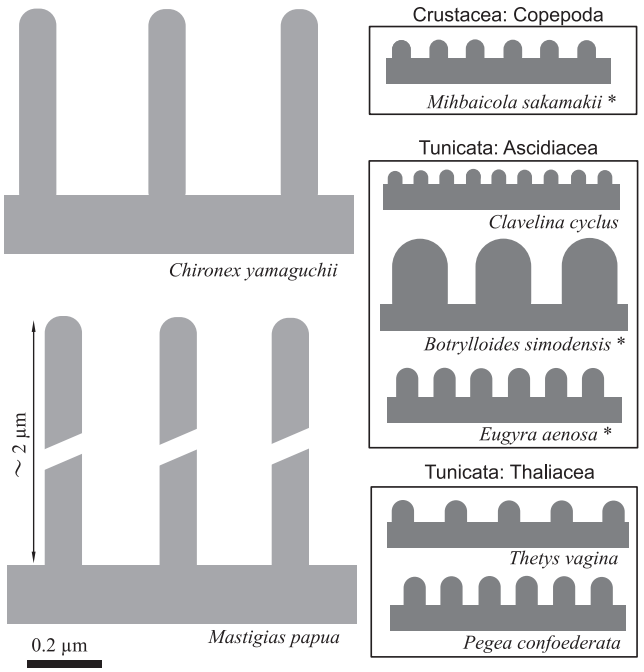

Fig. 8. Simplified schemas of the microvilli on the exumbrellar epidermis (light gray) and nipple arrays on the cuticular surface of a copepod, ascidian, and salp (dark gray). The length (height), width, and pitch of the cuticular nipple arrays were obtained by referring to Hirose and Uyeno (2014) for *Mihbaicola sakamakii*, Sakai et al. (2019) for *Clavelina cyclus*, Hirose et al. (1990) for *Botrylloides simodensis*, Hirose et al. (1997) for *Eugyra aenosa*, Kakiuchida et al. (2017) for *Thetys vagina*, and Sakai et al. (2018) for *Pegea confoederata*. Asterisks indicate that the dimensions of the nipple arrays were obtained by measuring the electron micrographs in the references.

pelagic species studied here, *Au. coerulea* and *D. chamissonis* do not have the array of microvilli on the exumbrellar surface. This may be because the functional properties potentially provided by the array would not be important for the species, or they may have countermeasures to compensate for the absence of the array. The fundamental functions of microvilli have been mainly studied for understanding molecular transport and cellular sensors in mammals (Lange, 2010), but the physical properties of the microvillar surface are also functionally important for the organisms.

The simulation of light reflection and diffraction in the simplified model of microvilli indicated that the relative reflectance is smaller than 1 in most pitches of pillars, supporting the anti-glare property of the microvillar array. However, the reflectance drastically fluctuates depending on the pitch of the pillars, and it is more than 1 at some particular pitches (e.g., open circle in Fig. 7B). The simulation showed that the height and pitch of pillars are important parameters determining the optical properties of the model. In the condition comparable to the microvilli of *Ch. yamaguchii*, the reflectance is one-third of the reflectance of the flat surface, indicating a remarkable anti-glare property. Similarly, in the condition of *M. papua*, the reflectance is half for TE wave and 70% for TM wave. Accordingly, the exumbrellar microvilli of *Ch. yamaguchii* and *M. papua* probably serve to camouflage epipelagic jellyfish. On the other hand, the refractive index of the exumbrellar epidermis was assumed to be 1.443 in the present simulation, considering the refractive index would not differ much from that of seawater. It is necessary to measure the refractive index of the exumbrellar surface for better evaluation of the anti-glare property of the microvillar array. It should be noted that pillars stand erect in the model for the simulation but the microvilli often bend. Additionally, the gaps among the microvilli were filled with mucus, as observed in *Ch. yamaguchii* (Fig. 3C). These differences potentially affect the properties of the microvilli, such as reflectance.

In conclusion, the present study showed variations in the exumbrellar structures of six cnidarian medusae, covering Hydrozoa, Cubozoa, and Scyphozoa distributed across both the epipelagic and mesopelagic layers. There were specific differences in terms of the shape and size of epidermal cells, presence or absence of microvilli, and the thickness of the layer of thickened mesoglea matrix beneath the epidermis. These differences probably reflect the different lifecycle strategy of each species. Additionally, the microvilli on the exumbrellar epidermis may provide functional properties for the body surface. The present observations raised the possibility of the natural absence of the exumbrellar epidermis in mesopelagic cnidarian medusae; however, it is still not clear if their exumbrellar surfaces are covered with the epidermis in natural environments. Further studies are necessary to better understand the structural diversity of exumbrellar tissues and their potential functions.

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COMPETING INTERESTS

The authors have no competing interest to declare.

AUTHOR CONTRIBUTIONS

AI, YO, JN performed the sample collections and the laboratory-culture, and DS performed the simulation of light reflectance. EH carried out ultrastructural observations and prepared the manuscript draft. All authors gave final approval for publication.

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