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How the Skin of *Octopus vulgaris* Makes the Animal Suitable for its Environment?

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ABSTRACT

The skin of Cephalopods has special patterns or characteristics that make animals suitable and better for survival in their environment and transforms behavior from a simple manner to complex. All authors addressed *Octopus* respiration through the skin, without detecting evidence for this emphasis. In this study, the author found this evidence, indicating the presence of the organ responsible for this process. The author introduced evidence via an accurate examination of the whole skin, especially the upper eye area. The epidermis of an *Octopus* contains minute tubes (first record) in the mantle regions and pores; they may be used in extruding mucus outside the skin and breathing in unsuitable environmental conditions or during the attack of the crustaceans outside the water upon detection. The animal would survive outside the marine environment by breathing the air from the external environment; this is common in many marine species such as Hagfish. The skin of *Octopus vulgaris* has a special formation in the epidermis which is covered by mucus and numerous types of mucus cell secretions, followed by an extensive dermis, with connective tissue from the areolar type for trapping the air under unsuitable circumstances. The multicolored and multilayer (first record) chromophores are the major components for concealment and camouflage behavior to change skin color. In addition, the photoreceptor cell in the upper epidermis is not similar to the deep goblet cell (first record), the mucus layer for protecting the animals from dryness during the animal attacks against their prey outside the water column. The epidermis followed by the wide dermis contains the cuticle in the upper dermis, it is positive for PAS stain. In the dermis, there are iridophores, a network of collagen fibers, blood sinus and hyaline cartilage in the *Octopus* dermis, respectively.

INTRODUCTION

Cephalopods like all soft-bodied animals and other aquatic animals obtain oxygen and perform gases exchange through their skin. This phenomenon is common in invertebrates and some vertebrates who depend on specific aspects to get oxygen through animal skin, among which are the cephalopods in unsuitable cases **(Wells, 1990; Anderson *et al.*, 2010; Birk *et al.*, 2018; Ajala *et al.*, 2022; Frey *et al.*, 2022)**. The epidermis is covered by mucus, except for some areas used for breath; mucus is used to protect the animals from the invasion of foreign material attached, parasites and dryness, adding to the existence of numerous papillae structures on the outer surface of epidermal cells. The dermis is extensive and composed of connective tissue areolar performed by collagen fibers **(Allen *et al.*, 2014; Naomi *et al.*, 2021; Imperadore *et al.*, 2022; Iskarous *et al.*, 2022)**. Moreover, the animal has multi-types of mucus secretion cells in the upper epidermis used for defense against foreign organisms whether antiparasitic, antifungal, antibacterial or antiviral ones; in addition, these cells protect from dryness. The *Octopus* dermis region is wide in section and includes a network of vascular collagenous fibers, chromophores below the iridocytes, hyaline cartilage among the collagen fibers, and retractor muscles that give the skin papilla **(Allen *et al*., 2014; Stubbs *et al.*, 2016; Krishnamoorthi *et al*., 2017)**. At the beginning of the early stage, the animal breathes with skin, and is a common invertebrate hatching. During the evolution of Cephalopod, the animal loses the external skin which is replaced with a pair of lungs to become suitable for the environment **(Chitty, 2022; Lo Presti *et al*., 2022; Stanisic *et al*., 2022; Strand *et al.*, 2022; Tunnah *et al*., 2022; Vargheese *et al*., 2022)**. Cephalopods are the masters of rapid coloration changeable to attract a mate; sometimes animals use this potential for communications, fear others, as well as escaping from predators **(Williams *et al*., 2019; Al-Soudy *et al*., 2021; Ikeda, 2021, Ishida, 2021; Jung, 2021)**. Changing color results from the presence of special structures in animal skin, which has a special structural nature, such as multi-layers chromophores, melanophores, lecucophores and iridophores **(Gonzalez-Costa *et al*., 2020)**. The hyaline cartilage is a connective tissue and functions in supporting the organs of the animals such as the skeleton and is very important in the evolution of animals, maybe originating from the collagen fibbers network (**Krishnamoorthi *et al*., 2017; Avila Rodriguez, 2018; Irawan *et al*., 2018; Anadón, 2019; Al-Soudy *et al*., 2021; Naomi, 2021; Liu *et al.*, 2022; Lukeneder *et al*., 2022)**. Some organs as **t**he *Octopus* dermis has different types of cuticle glands which secrete semi- hard carbohydrate cuticle, and the cell is positive for carbohydrates stain. In addition, protein stain are positive as melanocytes, and all secretory cells contain neutral and melanin ones **(Lee *et al*., 2014; Birk *et al.*, 2018; Anadón, 2019; Fernández-Gago, 2019; Gestal *et al.*, 2019**; **Dill-Okubo *et al*., 2021)**. Some authors didn't find the linkages between the breath by skin, and the presence of animal outside the water, changing in color rapidly, fast swimming process in this phylum and their body form. In Cephalopod, the animal has evolutions in many systems of bodies, as color changing for concealment and the presence of two stomachs in the digestive system. Furthermore, the animals have an advanced defense system including the ink gland and the anterior and posterior salivary glands **(Gonçalves *et al.*, 2021; Ikeda, 2021; Abd Elrheem, 2022)**. The previous structure in this study related an important role by coordinating each other to make the animals survive in their environment by an evolution process.

From the above, we can say that the goal of this study was to discuss in deep detail the skin structure of *Octopus vulgaris* caught from the Red Sea, Egypt via many methods.

MATERIALS AND METHODS

2.1. Collection

Samples of *Octopus vulgaris* were collected from a selected site on the western coast of the Red Sea, Egypt. This site is 17km south of Safaga (latitude 26° 38' N longitude 33° 59' E). The collection site is on rocky shores, and the samples were collected from the intertidal zone at the time of low tide. The collection was done using the hand and were then packed up. One specimen was collected and put in a plastic container containing seawater. Specimens were anaesthetized by adding menthol crystal (El -Nasser Chemicals Company) to the water surface of the jar and waiting until the sample got relaxed. The specimens were dissected in the field to obtain the organs under study, which were fixed in Blouin's solution for 24 hours for histological preparations.

2.2. Scanning electron microscope

For scanning electron microscope (SEM) studies, the skin of *Octopus vulgaris* was fixed in a mixture of three volumes of 4% glutaraldehyde and one volume of 1% osmium tetroxide.

They were dehydrated in graded series of alcohol, critical point dried, gold-coated and monitored under a JEOL 5300 scanning electron microscope at an operating voltage ranging from 10- 30v.

2.3. Histological studies

Organs sectioned were cut off from the body and placed into Blouin's solution in seawater for 24 hours. Fixed parts were then passed to the graded series of alcohol from 30 to 100%. They were cleared in toluene three times each for 5 minutes, and then embedded in paraffin wax. Sectioning was made by microtome at 5- 7µm thickness. Sections were stained with the following stains:

Harris hematoxylin and eosin (H&E) combination **(Steedman, 1950)**;

Periodic acid Schiff's reaction (PAS) for demonstration of polysaccharides in various cells and tissues **(Carleton *et al*., 1967)**;

Masson's trichrome stains for the determination of general collagen **(Brury *et al*., 1980)**, and Mercuric Bromophenol blue for the demonstration of general proteins **(Mazia *et al*., 1953)**.

The slides were dehydrated through an ascending series of ethanol after staining. They were then passed through xylene to a mounting medium and covered with coverslips.

RESULTS

Octopus vulgaris as a typical species is present in the Red Sea, Egypt, and it is widely distributed.

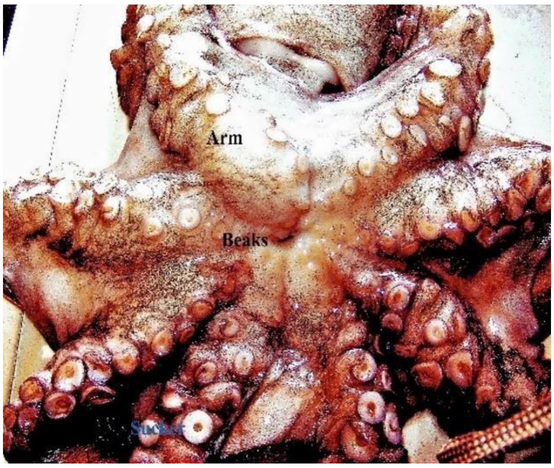


Fig. 1. The body plan of a marine *Octopus vulgaris* (Cuvier, 1797). (Ventral view).

By scanning the electron micrograph, the skin of *Octopus vulgaris* of the outer epidermis seemed to have a very active tissue as the epidermis papilla appears in both light and micrograph as highs and lows, with some free mucus which appears as shine in the micrograph and a small tube between each compartment of skin. The epidermis contains skin with a small canal arriving in chromophores using it in mucus extrusions outside to cover the body of animals, which appears so shine in micrograph (Figs. 2A, B). In the mantle in the upper eyes region, the animals have six-minute pores arranged in two rows, free of mucus so they are dark in the micrograph, which may be used for breath during the unsuitable substance as absence or decreasing oxygen manner or for trapping the air to areolar tissue of demise

(Figs. 2C, B). The epidermis contains a minute cuticle structure scattered on the epidermis to support the loose skin, which appears in the micrograph as a cuticle manner recorded in all previous literature, shining in micrograph in a leave plant shape and may be used for supporting the loose or soft epidermis and appears clear. The pore is used for consuming the dissolving oxygen in the water and trapping the external air to the water. In the external environment, animals predate their preys as crabs outside the water (Fig. 2D).

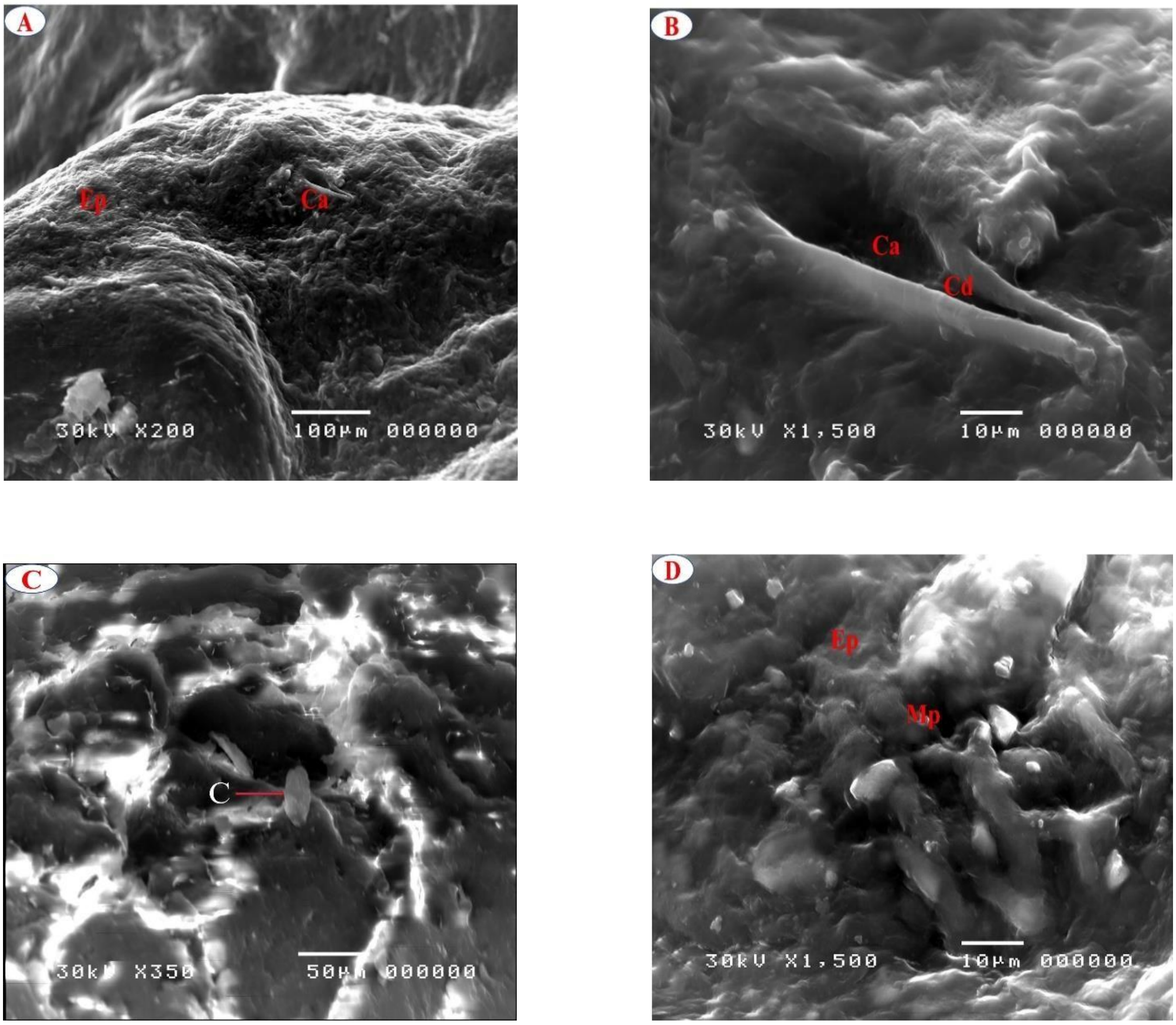
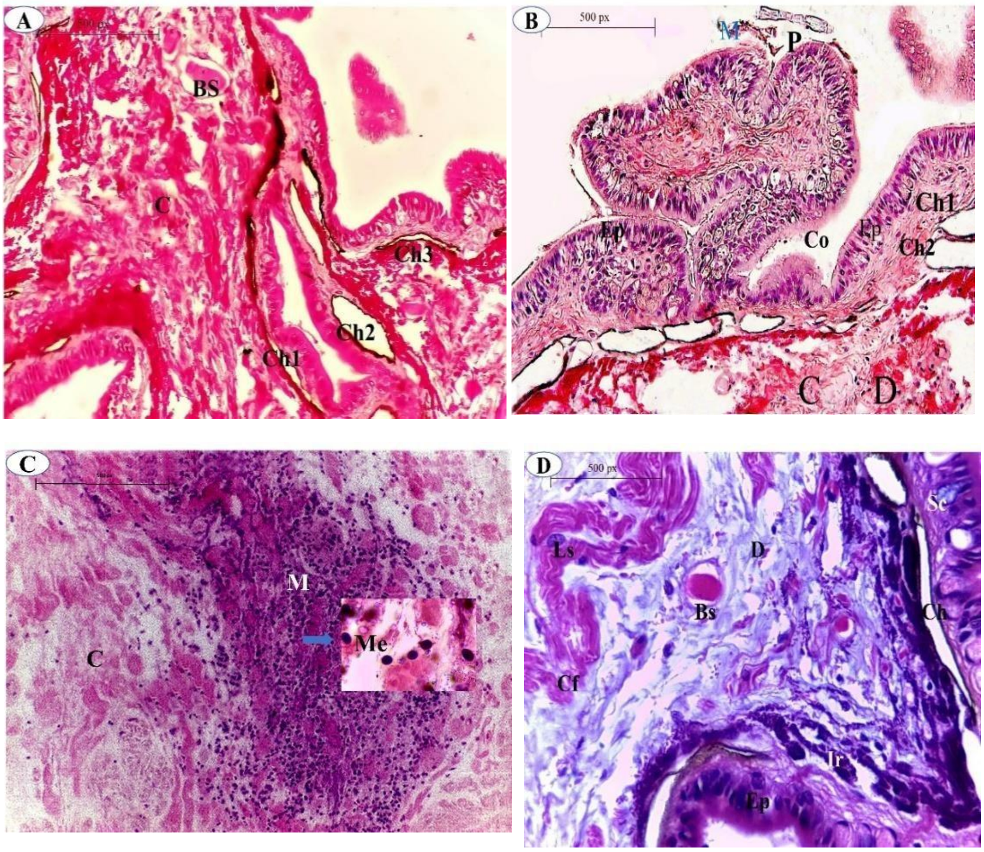


Fig. 2. Scanning electron micrograph (Ca), cuticle canal, (Cd) cuticle duct, (C), cuticle, (Ep), epidermis, (Mp) and mantle pores.

By examination, the section which is stained by H&E, the skin of *Octopus vulgaris* has a marvelous pattern forming papilla (P) as rapid and highly coordinated with optical visions manners. The skin epidermis of animals is covered by a mucus layer (M) to protect animals from parasites and dryness at low tide. The epidermis consists of columnar epithelium, and the structure of the chromophores cell gives different colorations to achieve vastly, and sufficiently different appearances by chromophores. The author noted multi layers of chromophores in the mantle region, arranging in three rows as the first record for giving the animals a continuous color, a nonstop color (Ch1, Ch2, Ch3) (Fig. 3.B, D, E, F). By (H&E) stains, the skin papilla (P) appears, and the deep goblet cells PAS positively (Gc) invade the skin, which is used for mucus secretion and gives the skin moisture and has neck opening outside the skin to the external side. Additionally, the sensory cell appears for the skin surface to achieve better sensation by clear skin for any amount of light (first record) (Sc) used by animals to differentiate between diurnal, nocturnal and shadow. It is sensitive to light and do not have any relationship between the goblet cells in function and secretion during high temperatures. The goblet cell has a basal nucleus, deep inside the epidermis, and has open for a pore to discharge its secretion outside the skin. The Sensory cell lays the upper epidermis in a higher layer of skin, to become suitable for the incident light. Also, the goblet cells are producing mucus and stain their content by PAS stain. Also, the presence of three types of cells all for mucus secretion to achieve better moisture for skin, the animals used in mucus secretion as previous goblet cells (M1, M2, Gc) (**Figs. 3, F, G)**. Much melanin fills the chromophore cell in the dermis are spread in sections. The melanin in chromophores upper dermis, and deep dermis of *Octopus vulgaris* appeared positive by (H&E) stain, bromophenol blue stain (protein stain) also, in the epidermis deep layer (**Fig. 3, C, J**). The connective tissue in the epidermis of *Octopus vulgaris* skin from spongy type for facilitating the animals trapped in the air between this tissue which is areolar connective tissue type and spread in the dermis (**Figs. 3, D, H**). The blood sinuses are spread between sections they appear by masons' trichrome stains, and hyaline cartilage (Hc) (**Figs. 3, H, I**).

Examining the whole skin under Binuclear Microscope appears the chromophores cells appear in the upper epidermis and have borders and pathways because they appear by the clarity of the skin. The chromophores are scattered in the skin and they are in three cases, free melanin, fill with melanin, and density of melanin. The first case takes place when animals are resting during the night, the second during stress, and the third case appear after collecting the melanin in chromophore cell during stress. The chromophores cell contain many pigment colors, they are arranged by nervous system control to achieve a suitable appearance.



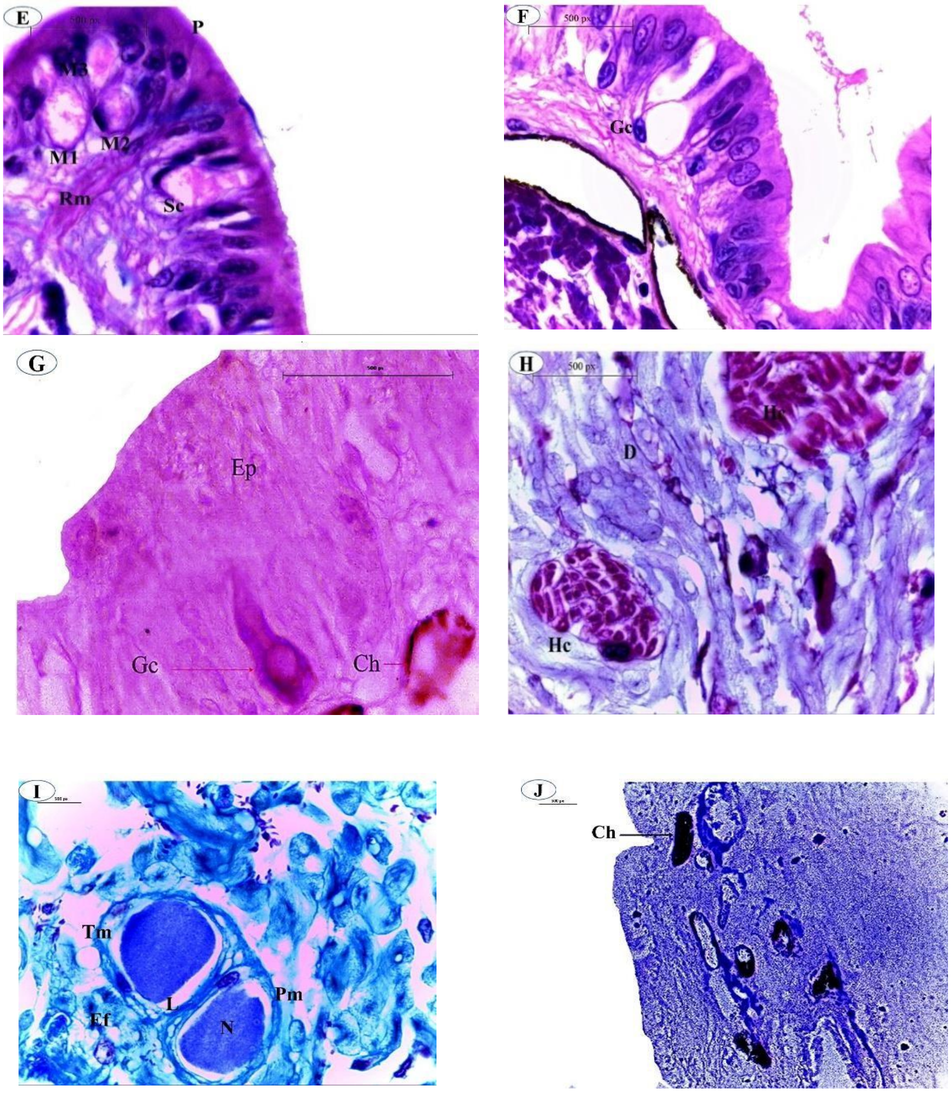


Fig. 3. Transversal section of the skin of *Octopus vulgaris* stained by (H&E), Masson trichrome, PAS, bromophenol stains shows: (Bs), blood sinus (E.P), Epidermis; (C.H), chromophores, columnar epithelium; (C), connective tissue, (Cf), collagen fibers, (D), dermis, (Ef), Elastic fiber, (Gc), goblet cell, (Hc), Hyaline cartilage, (Ls), collagen strands, (N) nucleus, (M), mucus, (Me), melanin cell, (M.F), muscle fibers (Sc), sensory cell.

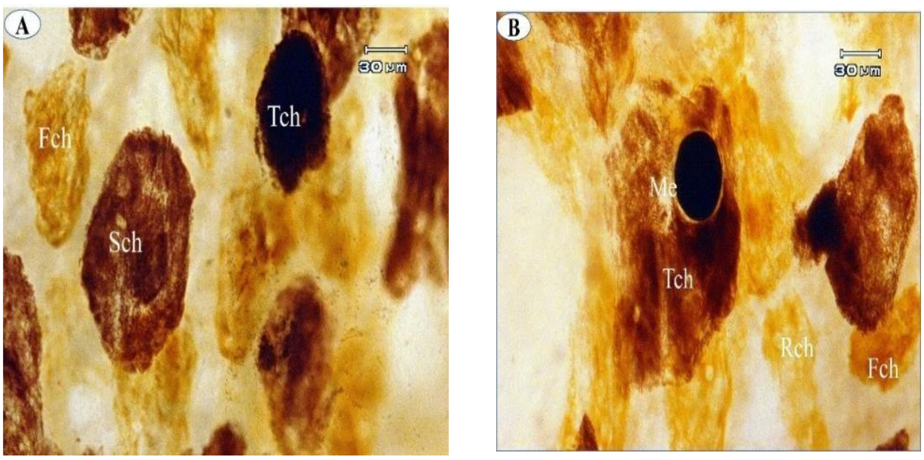


Fig. 4. Vertical section of skin under the binocular Microscope Show: (Fch), free chromophore Melanin, (Tch), twisted chromophore, (Sch) skin chromophore cell, (Rch), resting chromophore.

DISCUSSION

Scanning Electron Micrograph investigation on the mantle skin of *Octopus vulgaris* the Epidermis presence pores and canal like a small tube. Some authors have been studying the unsuitable condition surrounding the *Octopus* and suggested a present mechanism for air exchange these results agree **(Wells, 1990; Birk *et al.*, 2018)**. The animals uptake the oxygen through the skin in some cases decreasing the oxygen content, outside the water when attacking the crab. The examination of the skin by scanning Electron micrographs and by different stains found more evidence supporting this emphasis **(Booth and Feder, 1991; Melzner *et al.*, 2007; Birk *et al.*, 2018).** The skin of *Octopus vulgaris* contains many pores these pores may be used in helping air exchange between animals and the external environment, areolar connective tissue, and cuticle resemble evidence **(Birk *et al.*, 2018; Gül *et al.*, 2022).** The epidermis of the Octopus as a common invertebrate consists of columnar epithelium cells containing many mucus secretions cells and photoreceptors cells **(Gehring, 2004; Fichi *et al.*, 2015; Kingston *et al.*, 2015; Stubbs, 2016; Fernández-Gago *et al.*, 2019; Anadón, 2019, and Hanovice *et al.*, 2019).** On the upper surface of the epidermis presence of mucus layers and sculptures killed papilla agree **(Allen *et al.*, 2013; Allen *et al.*, 2014; Birchenough, 2015; Gonzalez-Costa *et al.*, 2020).**

Immediately below the epidermis, there are layers (dermis) that possess a series of sacs with pigment. The multicolor and layer chromophores and Iridophores are typically brown and determine color production for the camouflage of animals agree with **(How, 2017; Reiter, 2018; Williams *et al.*, 2019; Imperadore *et al.*, 2022).** Other colors are attainable by using the second layer dermis structures in deep position melanin cells, the network of collagen fibers, and blood sinus, all-important contributions to body coloring **(Hanovice *et al.*, 2019; Williams *et al.*, 2019; Ikeda, 2021; Ishida, 2021).** The binocular microscope shows the skin of the cephalopods consisting of a transparent epidermis shows epidermal chromophores, free melanin pigment cells in the dermal layer of varying thickness formed outer dermis. The physiological color change is most widely studied in melanophores since melanin is the darkest and most visible pigment **(Reiter, 2018; Williams *et al.*, 2019; GonzálezCosta, 2020; Ikeda, 2021; Ishida, 2021).**

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