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Research article

The identification and distribution of the mucous secreting cells in the integument of the Schaap's dragonet, *Callionymus schaapii*, Bleeker, 1852

Sinlapachai Senarat[1], Piyakorn Boonyoung[2], Jes Kettratad[1], Wannee Jiraungkoorskul[3], Pisit Poolprasert[4,*], Shuaiqin Huang[5], Theerakamol Pengsakul[6], Ezra Mongkolchaichana[7] and Chamnan Para[8]

[1]Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330,Thailand
[2]Department of Anatomy, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand
[3]Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
[4]Program of Biology, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand
[5]State Key Laboratory of Cellular Stress Biology, and Parasitology Research Laboratory, School of Life Sciences, Xiamen University, Xiamen 361102, China
[6]Faculty of Medical Technology, Prince of Songkla University, Songkhla 90110, Thailand
[7]Department of General Science, Faculty of Science, Navamindrahiraj University, Bangkok 10330, Thailand
[8]Department of Western Languages and Linguistics, Faculty of Humanities and Social Sciences, Mahasarakham University, Mahasarakham 44130, Thailand

Abstract

The microanatomical structure of mucous secreting cells (Ms) in fish has been widely found; however, there is a lack of research on the Schaap's dragonet, *Callionymus schaapii*, which is regarded as an important benthic fish. The identification and distribution of Ms in different areas along the integumentary system of *Callionymus schaapii* were therefore demonstrated and compared using histochemical techniques. The integument system of this fish consists of two layers: an outer epidermis and an underlying dermis. In particular, the Ms can be classified because they were positively stained with periodic acid-schiff and alcian blue methods. As a result, the distribution of Ms could be observed in all areas (lateral head, pectoral – pelvic, middle trunk and caudal areas) and along with the integumentary system, respectively. The observation confirmed that the highest number of Ms was found in the pectoral-pelvic area. The caudal area had the lowest number of this cell. The localization and abundance of Ms in the integument may support the behavior and enhance swimming to the area under the estuarine conditions.

Keywords: Estuarine area, Integument system, Mucous secreting, Thailand, Schaap's dragonet

***Corresponding author:** Pisit Poolprasert, Program of Biology, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand. Email: poolprasert_p@psru.ac.th

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INTRODUCTION

The skin of fish is a prime organ and performs various functions such as being the primary protective barrier against noxious agents from environmental changes which is helpful to support survival and adaptation (Groman, 1982; Genten et al., 2009). The skin is histologically composed of three layers (from the outer to inner zones) including epidermis, dermis, and hypodermis (Groman, 1982; Genten et al., 2009). Some previous literature reported that the epidermis has several types of secretory cells, especially Ms (Sire and Akimenko, 2004; Ghattas and Yani, 2010). Moreover, the Ms has been continually described in many fish (Lewis, 1970; Mittal et al., 1994). Its large size, containing cytoplasmic vesicles, characterizes this cell. The Ms contained major constituents of chemical compositions such as glycoprotein with oxidizable vicinal diols and O-sulphate ester (Lewis, 1970; Mittal et al., 1994). The key roles of these compositions are as a natural defense against parasites and pathogenic organisms, locomotion, and lubrication of the fish (Palaksha et al., 2008). Additionally, several investigators have also hypothesized and discussed the potential role of this mucus cell. They concluded that the Ms may be responsible for the osmoregulation in the fish (Scott, 1989; Powell et al., 1994). This conclusion is similar to the previous study of Yuge et al. (2003) in that the secretion of the mucus cells was related to the luminal osmolality, water regulation and salt absorption. However, the characterization of Ms in Family Callionymidae has never been reported.

The Schaap's dragonet, *Callionymus schaapii* Bleeker, 1852, is collected as a representative species from the family Callionymidae. It is commonly found and the most abundant in the eastern Indian Ocean and the western Pacific Ocean (Randall and Lim, 2000). This species is known as a crucial part of benthic estuarine species and may directly process a significant portion of the estuarine benthic biomass (Randall and Lim, 2000). This raised the question of whether or not the Ms may be part of the change with existing natural environmental conditions in both natural and laboratory habitats. It is possible that this knowledge provides useful information to apply on behavior and physiological research.

However, there are wide rooms for a better understanding of this question; the current knowledge on this topic is still unknown, and little described due to the lack of histological data of the Ms. This study sets out primarily by identifying and describing the Ms of *C. schaapii* using histochemical techniques before further investigations.

MATERIALS and METHODS

Fish samples and histological techniques

This field study observed the reproductive biology of the economical estuarine fish. There were dead female specimens of *Callionymus schaapii* (n=7 with 6.20±0.95 cm in total length) donated from a professional fisherman in October 2017 from the Pranburi River estuary, Thailand (N 12°24'16.5" / E 099°59'20.2", N12º) where the salinity condition was 24.0 ppt. Because only dead specimens were used, this study was not required to have the protocol approval. All specimens were fixed in Davidson's fixative containing a mixture of 330 ml 95% ethyl alcohol, 220 ml 37% formalin, 115 ml glacial acetic acid and 335 ml distilled water (Dietrich and Krieger, 2009) at room temperature for 24 hours prior to a further study.

Due to the symmetrical body of this fish, only the part of the right body was used, which consisted of approximately 5 × 5 mm2 fragments harvested from four areas of the integument; lateral head, pectoral – pelvic, middle trunk and caudal areas; (Schematic diagram Figure 1A). Each fragment was processed using standard histological techniques according to Presnell and Schreibman (1997) and Suvarna et al. (2013). Paraffin sections were cut at a 4 µm thickness and histochemically stained with Masson's trichrome (MT). They were then brought to observe the fiber and connective tissue and periodic acid-schiff (PAS) to detect the glycoprotein, alcian blue (AB), and the mucopolysacharide.

The presence and localization of Ms in the integument were observed from the orientation of the histological sections and photographed with TE750-Ua microscope equipment. The distribution of Ms was rearranged by an adobe illustrator CS5. Additionally, the number of Ms was counted in all areas and then sampled from three slides. Each slide had 3 selected sections; all of them were determined as mean±SD. To explore the difference in number of Ms between areas of integument, ANOVA followed by Tukey's HSD tests were performed by using SPSS for windows version 25.0 (IBM Corp., Armonk, NY, US). Results were expressed in terms of mean±SD. A value of $P<0.05$ was also considered to be statistically significant.

RESULTS and DISCUSSION

The identification of the integument and mucous secreting cells (MSC)

The results demonstrated the histological structure of the integumentary system of *C. schaapii* in figure 1B. The structure itself consisted of two layers: an outer epidermis and an underlying dermis (Figure 1C). Results of the observation were similar to some fish species, such as *Gnathonemus petersi* and *Astronotus ocellatus* (Mittal and Munshi, 1970), and Bagarius bagarius (Harder, 1975). These results contrast to other fish, which have three principle layers: epidermis, dermis and, hypodermis (Mittal et al., 1980; Whitear, 1986; Park and Kim, 1999; Park and Kim, 2000).

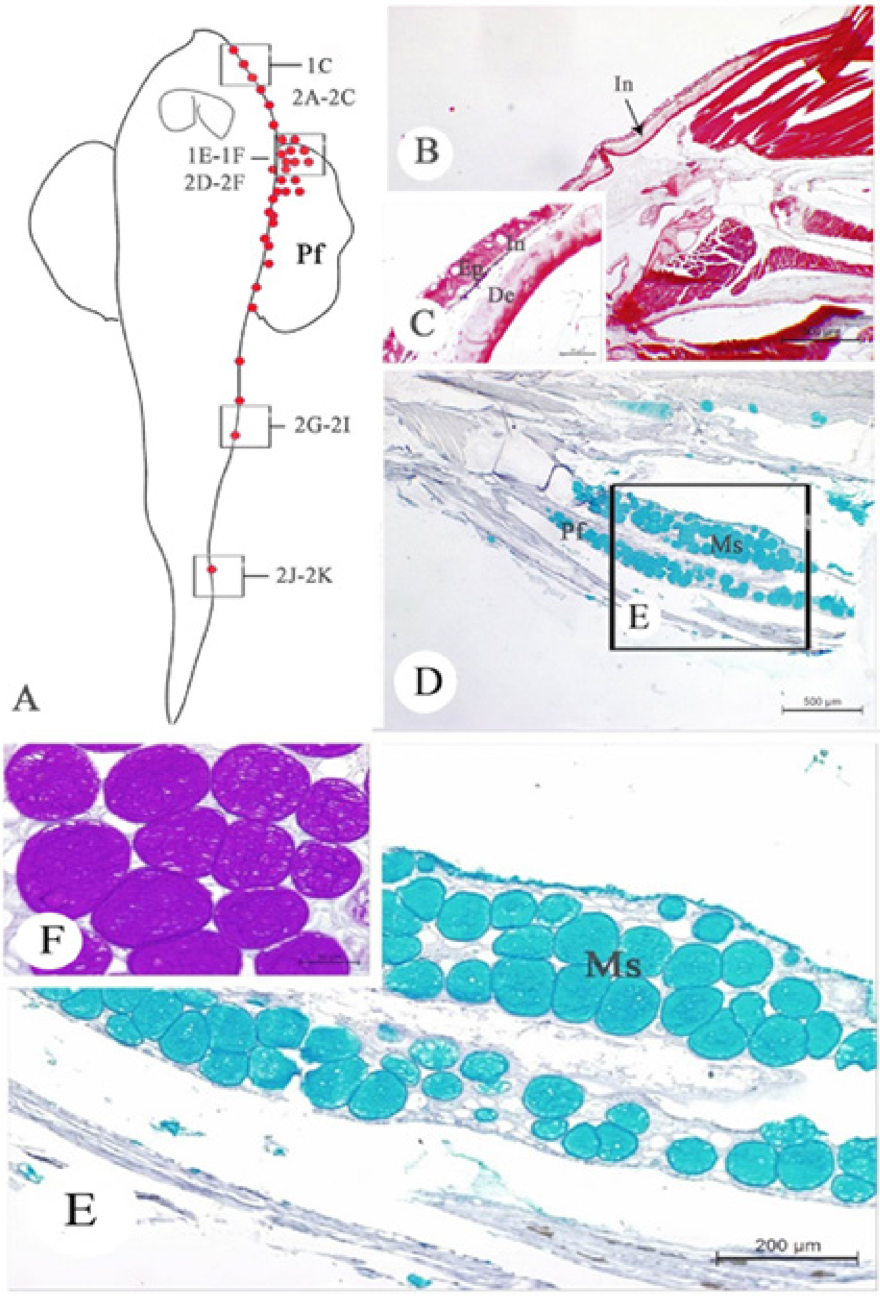

Figure 1 A: Schematic diagram showing the distribution of mucous secreting cell (Ms) along the integumentary system of *Callionymus schaapii*. B-C: Histological structure of the integument system (In) consisted of two layers including epidermis (Ep) and dermis (De). D-F: Histochemical techniques of Ms in the pectoral fin (Pf) positively stained with AB reaction (bluish, D-E) and PAS reaction (purple color, F). Abbreviation; Sse=stratified squamous epithelium. Note: B-C = stained with Masson's trichrome (MT), D-E = stained with alcian blue (AB), F = stained with Periodic acid-Schiff (PAS).

In these histological sections, oval/spherical shapes of Ms were observed in the superficial and middle epidermal layers. Although these cells varied in size with an average of 8 µm in height (range 10–60 µm), they did not histochemically differ. Some studies further added that the Ms in the middle layer of integument was the sacciform granulated cell/club cell (Agrawal and Mittal, 1992; Genten et al., 2009). The emptied Ms was negatively stained with H&E method (Data not shown); however, it was positively stained with AB (Figure 1E) and PAS methods (Figure 1F). This indicated that the presence of the chemical compositions (glycoproteins and mucopolysaccharide), were analogous to the Ms of *Acipenser gueldenstaedii* and *Pangio kuhlii* (Shephard, 1993; 1994, Genten et al., 2009). Previous ultrastructural observations suggested that the production of the glycoprotein was produced from Golgi cisternae, rough endoplasmic reticulum, nuclear envelopes, and plasma membranes in the Ms (Neutra and Leblond, 1966; Mittal et al., 1994). The proposed functions of these chemical compositions involved in the regulation of the respiration, the moisturization of the body surfaces, the defense against pathogenic microorganisms (Shephard, 1994; Sayer, 2005; Mazlan et al., 2006) and osmotic ionic regulation (Shephard, 1994) and stress protection as well as predator avoidance (Genten et al., 2009)

The distribution and comparative muti-localizations of mucous secreting cells

Another intriguing finding showed that the Ms was distributed throughout all tested areas (Figure 2); however, the number and the density of this cell varied in different areas including the lateral head (Figures 2A-2C), pectoral – pelvic (Figures 2D-2F), middle trunk (Figures 2G-2I) and caudal areas (Figures 2J-2K)

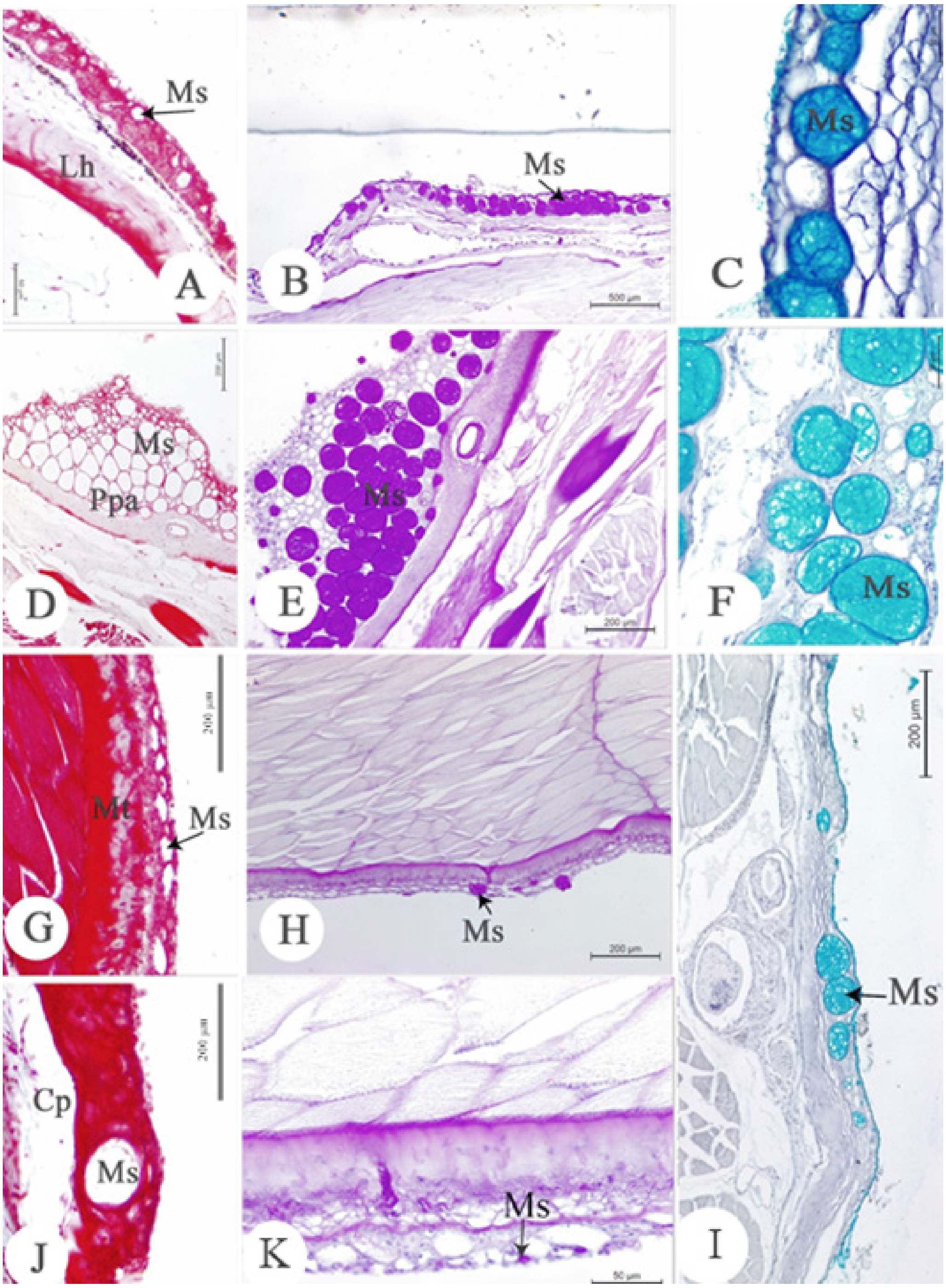

Figure 2 Light photomicrograph of the localization and distribution of mucous secreting cell (Ms) in different areas along the integument of *Callionymus schaapii* including lateral head area (Lh, A-C), pectoral-pelvic area (Ppa, D-F), middle trunk (Mt, G-I) and caudal part (Cp, J-K).
Note: A, D, G, J = stained with Masson's trichrome (MT), B, E, H, K = stained with Periodic acid-schiff (PAS), C, F, I = stained with alcian blue (AB).

The highest number (21.2±1.57 cells) of mucus secreting cells was observed in the pectoral – pelvic area, whereas the lowest density (1.4±0.50 cells) of this cell occurred in the caudal area (Figures 3). Based on the analysis of variance (ANOVA) F-test, there was a significant difference in means between the areas of the integument (F(3,36)=759.505, $P<0.05$). Besides, differences between means using the Tukey's HSD tests were also considered significant at the 95% level of confidence. The results indicated that means with the different letter were significantly different ($P<0.05$) as demonstrated in figure 3. Despite the number of limitations in this current study, the findings are relatively robust, but there is still some room for further studies to investigate. In addition, the high production of chemical components was observed in the pectoral – pelvic area, as a major site of the mucous integument. Because the pelvic fin of this fish contacts the sandy bottom in shallow estuarine habitats and mud flats (Randall and Lim, 2000), its fin may have a non-specific response and probably play a vital role in multiple biological activities, for example, the lubrication and protection of fish against abrasive injuries by maintaining suitable distance from the sandy bottom. In congruence with the study of Zaccone (1980), the mucous skin in the fish can help to reduce the friction within the water and was supportive of the swimming to a favorable area. This hypothesis on the precise roles of the Ms needs to be substantiated. However, the number of the Ms is based on the different factors such as temperature, season and stressor, and responses to fish mucous protection (Mittal et al., 1981). For example, the exposure to the acid environment on *Ictalurus nebulosus* skin related to the appearance of the hyperplasia of the Ms, implying an increase of the mucous production (Zuchelkowski et al., 1981). Also found in the previous observation, the drastically increased number of Ms in Dicentrachus labrax from exposure to chronic environmental hypoxia and high level of nitrate in the water was documented (Vatos et al., 2010)

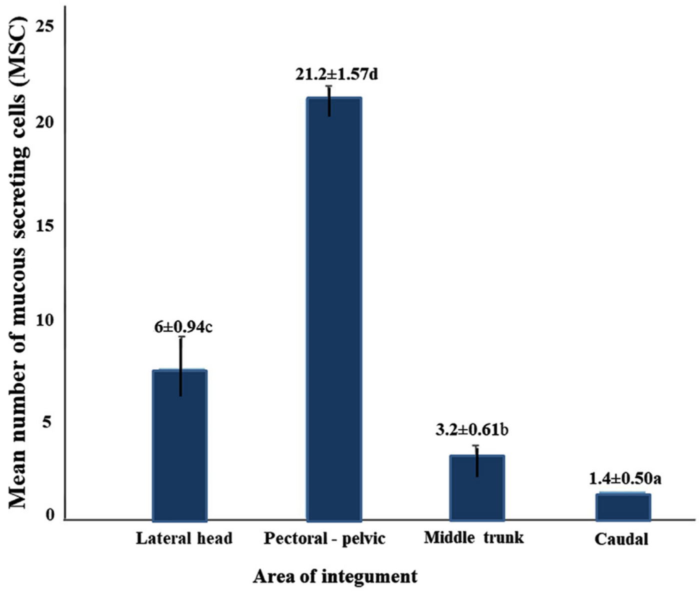

Figure 3 Histogram displaying the mean number of mucous secreting cells (Ms) in different areas (lateral head area, pectoral-pelvic area, middle trunk area and caudal area). The values represent as mean±SD and the mean difference is significant at the 0.05 level.

CONCLUSION

This present study is considered as the first report which revealed the presence of the Ms in *C. schaapii*. It demonstrated that the Ms was distributed along the integument. The highest number of Ms in the pectoral – pelvic area could have a tangible link to adapt to their environment under the estuarine conditions. In addition, the above findings become somewhat clear that the integument and characterizations on the Ms of *C. schaapii* could provide more information of the family Callionymidae.

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