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IDENTIFICATION OF THE PRIMARY AND SECONDARY MOULTING STAGES IN THE SHRIMP *METAPENAEUS AFFINIS* (H. MILNE-EDWARDS, 1837) BY THE RECOGNIZE CHANGES IN THE UROPOD

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Abstract

The present study examined the diagnosis of the basic and secondary stages of moulting cycle in commercial shrimp *Metapenaeus affinis* (H. Milne-Edwards, 1837). The moulting stage was classified to the five basic stages and sub stages. Postmoult stage A consisting of sub stages A1 and A2, and stage B sub stages B1 and B2; intermoult, stage C sub stages C1 ,C2, C3 and C4; premoult, stage D consisting of substages D1, D2 and D3/D4 and ecdysis, stage E are described and illustrated. The median part of the setae of inner uropods was examined by light microscopy. Bases of uropod setae, setal lumens, setal matrices, and setal cones showed remarkable changes during molting cycle from substages D1 (early premoult) to stage B (post moult), with distinct variations in epidermal retraction, pigmentation and setal development.

***Keyword*:** Moulting cycle, setae cons, premoult, postecdysis, commercial shrimp *Metapenaeus affinis.*

Introduction

The jinga shrimp*, Metapenaeus affinis* (H. Milne Edwards, 1837), occupy the "Gulf" and the Arabian Sea from the Gulf of Oman to South India (Khodanazary, 2019). It is also present in Sri Lanka, Philippines and Taiwan Island (Saoud *et al*., 2014). It is found in the depths of about 55 m (occasionally in deeper water to 90 m) from the coastline, mainly on mud or sandy-mud. The northwest of Persian Gulf is one of the traditional fishery activities in Iran and Iraq coastal region, where the most significant fishery resource rely on the benthic environment. Study of growth and development the indigenous species in aquaculture will help to the management the selective breeding programs, because the shrimps are important as the most consumed fishery products due to high demand for them in the world markets (Saoud *et al*., 2014). Shrimp is considered to be of the arthropods that are characterized by have an external, segmented skeleton (exoskeleton) composed mainly of chitin.

Growth and develop require that the exoskeleton be shed periodically to allow the body to expand (Stevenson, 1972). Which are essential to their evolutionary success (Promwikorn *et al*., 2004). The moulting process, termed ecdysis, is accomplished cross an innate sequence of behaviours and stereotyped motor activity, precisely proportionated by the interaction of steroid moulting hormones (ecdysteroids) and a complex suite of interacting neuropeptides (Shokoohmand *et al*., 2018). Molting plays an important role in development of crustaceans, in their growth, and in their reproduction. During their lifetime, crustaceans undergo precise physiological processes of molting, degrading the old exoskeleton, and synthesizing a new exoskeleton.

Several studies have been conducted on *M. affinis*, such as, a study shows Abdul-Sahib and Sultan (1996) possible relation of the moulting of *M. affinis* with the tidal phase was investigated. Abdul-Sahib and Ajeel (2005) studied the comparisons in the biochemical compositions (proteins, lipids, carbohydrates, ash) and caloric contents in the head, flesh and exoskeleton of the males and females of the commercial shrimp *M. affinis*. In the study of Saud *et al*. (2014) dealt with the chemical composition of species, *M. affinis* (H.Milne Edward), *Parapenaeopsis stylifera* as it gives great importance to know the nutritional value of the species and also reflects the physical condition of the species. Al-Maliki and Al- Khafaji (2018) studied the use of marine shrimp as a bio-indicator of heavy metal pollution. Also, it was studied in environmental monitoring investigations, and the human consumption of contaminated shrimp on human health.

Xue *et al*. (2017) studied the moulting in crustaceans, mainly controlled by ecdysteroids. It is assumed that, during the moulting cycle of crustacean, MIH secreted from the X-organ/sinus gland complex inhibits the ecdysteroid synthesis in Y-organs and suppresses molting. In crustaceans, MIH and members of the family of crustacean hyperglycemic hormone (CHH) neuropeptide, including crustacean hyperglycemic hormone (CHH) and gonad inhibiting hormone (GIH), have been widely investigated. These hormones are involved in many biological processes, such as moulting, reproduction, glucose metabolism, morphogenesis and osmoregulation. Moulting is a complex process, affected by a range of environmental factors and regulated by a cascade of hormonal signals involving changes in gene expression, cellular commitment, mitotic and secretory activity, endocrinology, behavior, and cell death (Kuballa and Elizur, 2007). This type of shrimp is considered one of the economic animals in our regional and local waters and its growth is one of the important factors in the success of the aquaculture process. The aim of the present work was throw light on the morphological changes in uropod setae during moulting to be used as criteria for moult staging in the shrimp, *M. affinis*,. Moreover, to provides a simple and practical evidence for the identification of the moulting substages in this well known as an important agricultural and commercial export product of Iraq.

Materials and Methods

Experimental animals

Specimens of the freshwater shrimp, *M. affinis*, were collected alive for this study from the River Al masahab East South of Al Hammar marsh Basrah Governorate. Part of the samples were transported to the laboratory. Shrimps were immediately acclimated and kept in two 1 L plastic bins. The adapted animals were transferred to their capacity ponds (60 x 60 x 30 cm) with recirculating system and two aerators for each bin and the category was approved for samples by length (5.5 – 8.5 cm) to monitor the phase-out moulting stages within 24 hours.

During the experiment, salinity was maintained at 35 % both in the bins and aquaria and temperature ranged from 28 – 30 ºC. The experimental animals were fed with commercial prawn pellets at 10% total body weight divided into 2 daily feedings. The waste was siphoned from the aquaria once every two days. They were weighed to the nearest 0.01 gm using an electric balance with an accuracy of 0.01 g after blotting excess water with absorbent tissues. The total body length, were measured with a Caliper Vernier with an accuracy of 0.01 mm.

Determination of morphological changes in uropod setae

Uropods of the *M. affinis* were examined and photographed under a light microscope (Olympus BX40) connected to a digital camera (DCE-2). The criteria used for moult staging were used according to Promwikorn, *et al*. (2004). For *P. clarkii* individuals molting cycle were classified into five stages according to Drach (1939), modified by Warner (1977) and Lowery (1988).

Results and Discussion

The characteristics and duration of the moulting stage after ecdysis in laboratory of *M. affinis* were investigated. The results showed remarkable morphological changes in uropod setae during different moulting stages and sub-stages as following: The setal development (setogenesis) and retraction of epidermis from the setal bases (apolysis) of the inner uropod near the telson tip gave a clear indicator of the moult cycle of M. affinis. Based on these criteria, the moult cycle of *M. affinis* could be categorized into four stages: postmoult (consist of sub-stages A and sub -stage B), intermoult (stage C consist of sub-stages C1, C2, C3,C4), premoult (stage D consist of sub-stages D0, D1, D2, D3/D4) and ecdysis (stage E). Although setogenesis has been used as a criterion for moult staging for many years (Drach, 1939). Species variations in setal morphology and development result in differences among crustaceans in both staging criteria and in easily-defined subdivision of the moult stages.

Postmoult (stage A and B)

In stage A (early postmoult), the whole body and exoskeleton were very soft and slimy, new setae are clear, with less distinct lumens, bases and nodes. Epidermis faint, with less dense pigments. Stage A could be divided into two substages, A1 and A2 (Figure 1, A1, A2). Substage A1 began soon after the prawn had flicked clear of the old cuticle. Setae were also very delicate. The setae and setal bases were filled with cellular matrix. The setal cone could not be seen. The setal node was poorly developed. Substage A1 lasted 1 hour after moulting in the animal experiment. The prawn entered the sub-stage A2, when the cellular matrix started to retract from the distal end of the setae. The cuticle was still soft to touch but not slimy. Constriction of cellular matrix continued until only the distal half of setae was filled. Setal bases were still filled with cellular matrix. The setal node was more developed, however, hardening of the exoskeleton had begun especially in the carapace and the sixth abdominal segment. At this stage, the entire body and the exoskeleton were found to be very soft, slippery to the touch, and pale in color, with a flesh-like appearance. Due to these characteristics, the prawns exhibited hiding behavior to avoid predation by other intermoult prawns, and an absence of internal cones was observed. The protoplasmic matrix filled the setae and setal bases According to Rusaini and Owens (2011) and Smith and Dall (1985) in *P. esculentus*, when the secretion of the endocuticle and the retraction of the cellular matrix began, the exoskeleton lost its slippery feel.

In stage B (late postmoult) Setal lumens are filled with setal matrix, epidermal pigments are dense and darker. This stage was marked by withdraw of the cellular matrix until the proximal half of the setae was emptied and was easily known by the clear space present in the base. Also stage B was marked by the formation of setal cones. Formation of setal cones was indicated by the constriction of cellular matrix at the base of setae (Khodanazary, 2019). The beginning of cuticular node development marked the B stage which generally took place 1-3 days after ecdysis. The exoskeleton became relatively hard, but it was easily depressed.

Intermoult (stage C) started after one day of moulting, this stage characterized by complete formation of setal cone arranged in one row at the base of setae, also the setal lumens were almost empty, and without pigment retraction. The exoskeleton achieved maximum rigidity in this stage. The most significant observation of stage C was that of the fully developed cuticular nodes and the formation of internal cones in the seta of the pleopods. The absence of cellular matrix within the setae were the most striking features that could be observed in intermoult stage (stage C) (Promwikorn et al., 2004; Shokoohmand et al., 2018).

Premoult (stage D) this stage was the longer moulting stages and divides into four substages as following:-

Substage D1 Setal lumens are clear without setal matrices (Figure 3) further withdrawal of epidermis from the setal bases was recognised by a clear zone between the setal bases and epidermis (Figure 3, D1). The clear zone marked the formation of new cuticle due to subsequent development of the new seta. Pigments are being retracted within epidermal layer at this substage. (Figure, 3). Similar patterns were found in *P. esculentus* (Shyamal *et al*., 2018). A straight line below the setal bases as a result of epidermal withdrawal marked the end of stage D0 (Romano, 2006). These characters were similar to that described by Promwikorn *et al*. (2004) on the black tiger shrimp (*Penaeus monodon*)

Substage D2 This stage was known by the area of the white area increases between the setal bases and epidermis, and the beginning of the pigment agglomerate Pigments of epidermis are variable in appearance (Figure, 3). Setal nodes started to impair. This stage could be observed from 7 to 12 d after moulting.

Substage D3 was characterized by the appearance of light pinpoint in the new setal nodes when examined under the

light microscope. This stage was observed 10 – 12 d after moulting in the experiment, the white area is very clear and the cones of the setea are irregular and the line is black between the cones of the setea and the epidermis (Figure 4)

Substage D4 At this substage, intense pigment withdraw from old cuticular setae was observed. Unusual increasing in wavy- like edge at proximal border of epidermal tissue appears. The pigment appears in parallel and dark color within the epidermis. (Figure 4). New setae were distinctly observed parallel to border of epidermal tissue. When the prawns entered the stage D3/D4. This stage could not be separated into two different moult stages due to the requirement for the experimental animals being sacrificed at every sampling time for another experiment (Saoud et al., 2014). The present results showed that, during premolt substages (D1-D4) epidermal pigment retraction, wavy-like edge of epidermal tissue and clear zone between epidermal tissue and old setal bases were distinguished which in agreement with those results reported on different decapod crustaceans (Chang *et al*., 2012; Oliphant *et al*., 2018; Vijayan *et al*., 1997; Wittmann *et al*., 2018).

Moult

Setal lumens are being filled with setal matrix. Epidermal pigments are faint and less dense comparable with other previous subtsages (Figure, 5). This stage is characterized by the appearance of new setea within the epidermis, and lack of cones. The last stage observed was ecdysis (moulting). The prawns shed the old exoskeleton and extended the new setae of the new exoskeleton. At ecdysis, the plastic exoskeleton was stretched by water uptake and an expanded haemocoel (Amer *et al*., 2015)

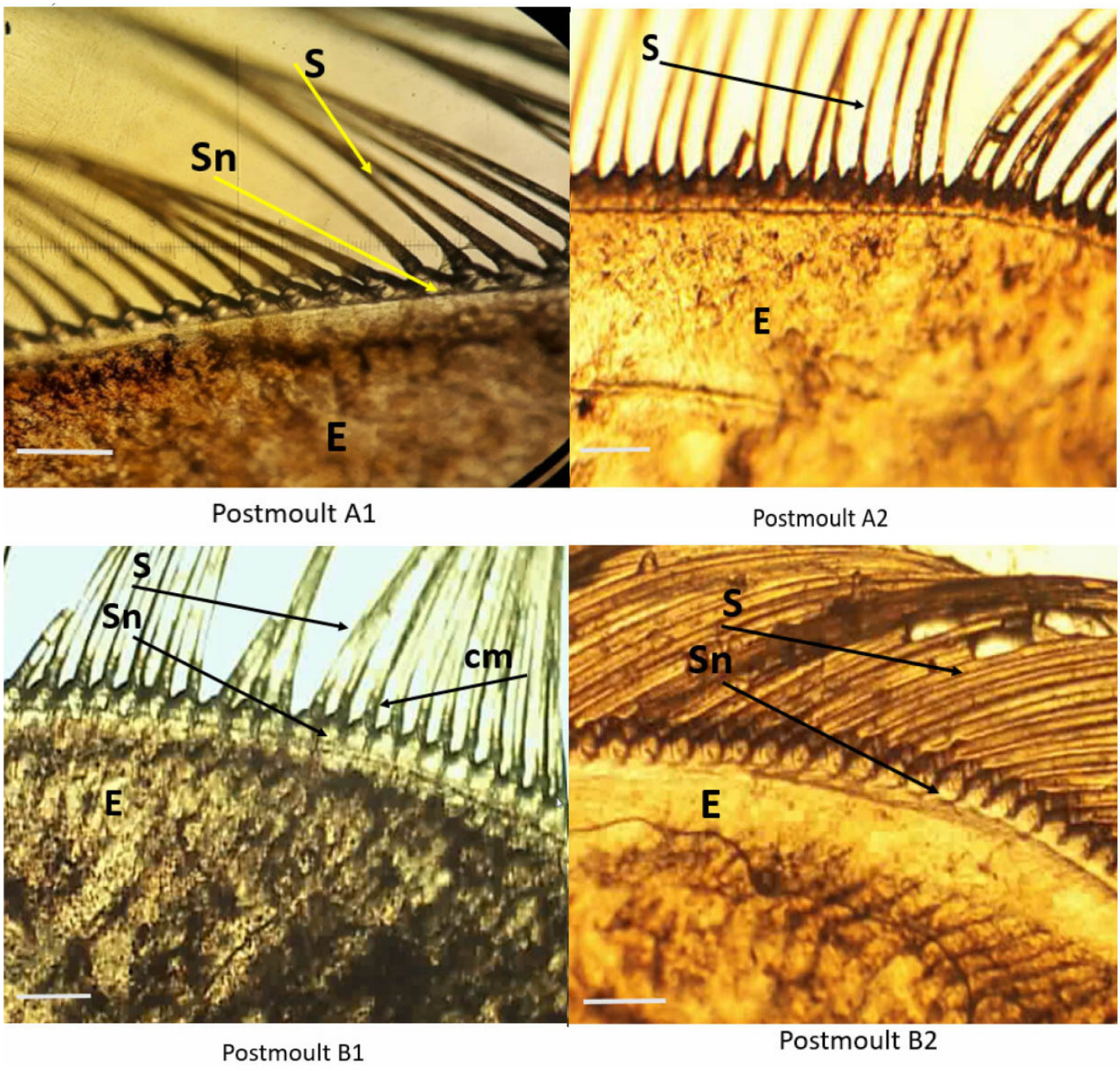

Figure 1: Ventral view of uropod setae postmoult stage A- B with magnification of 40X (A), and (B

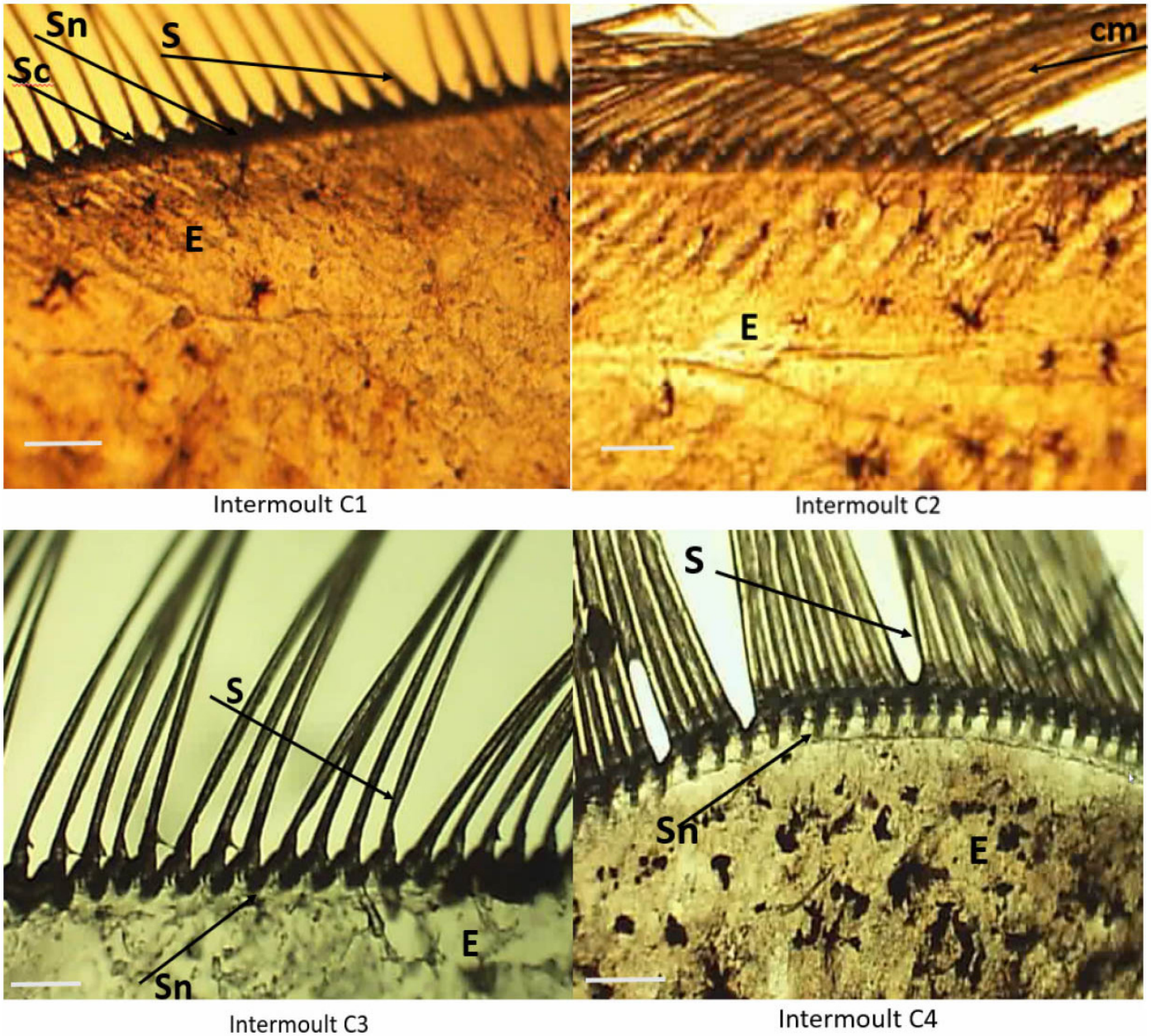

Figure 2: Ventral view of uropod setae stage C with magnification of 40X

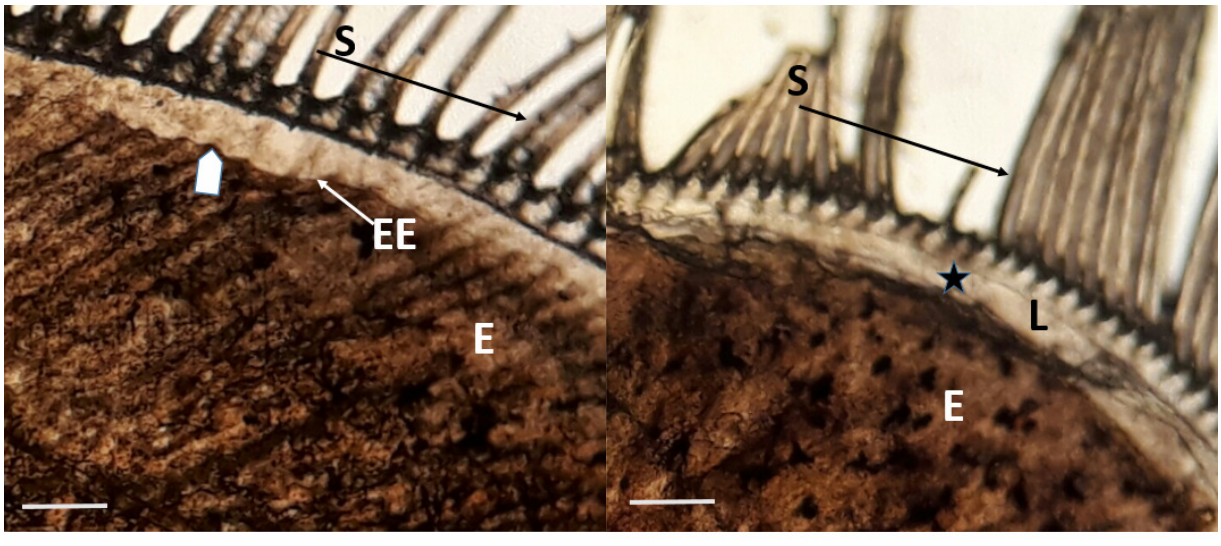

Figure 3: Ventral view of uropod setae premolt stage D1 and D2 with magnification of 40X.

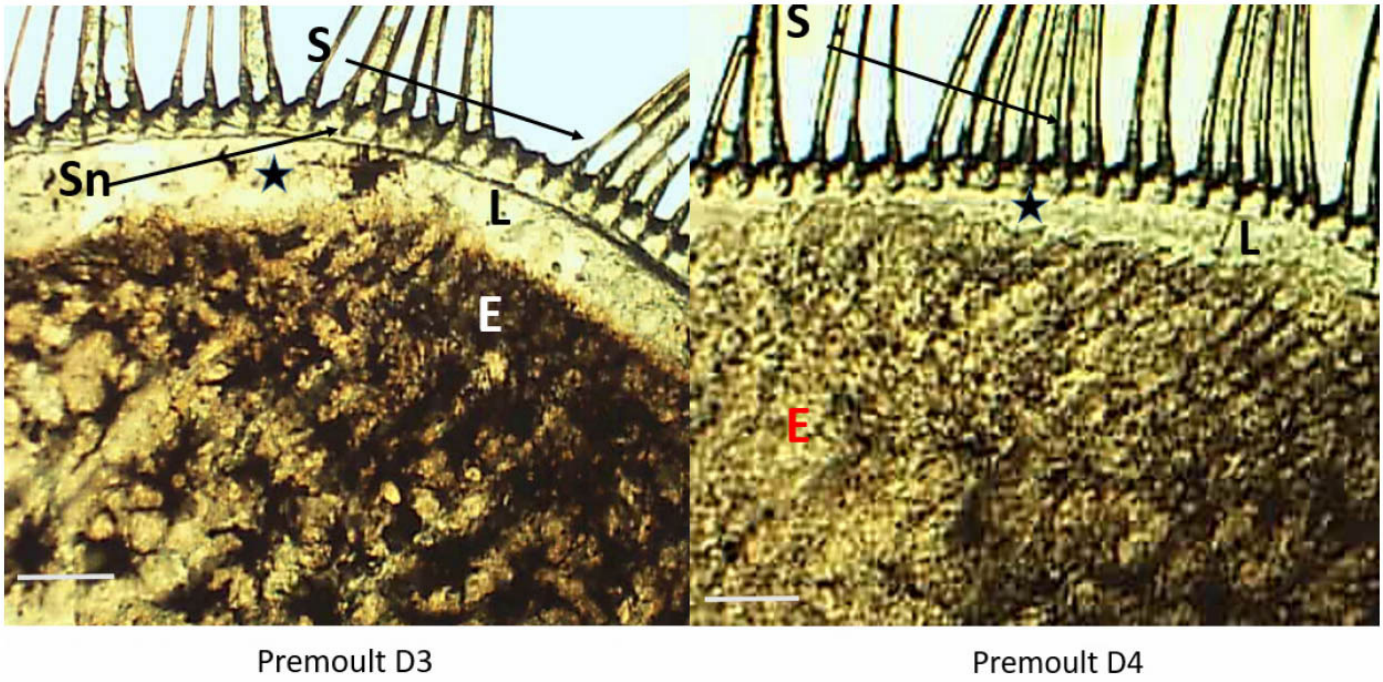

Figure 4: Ventral view of uropod setae premolt stage D3 and D4 with magnification of 40X.

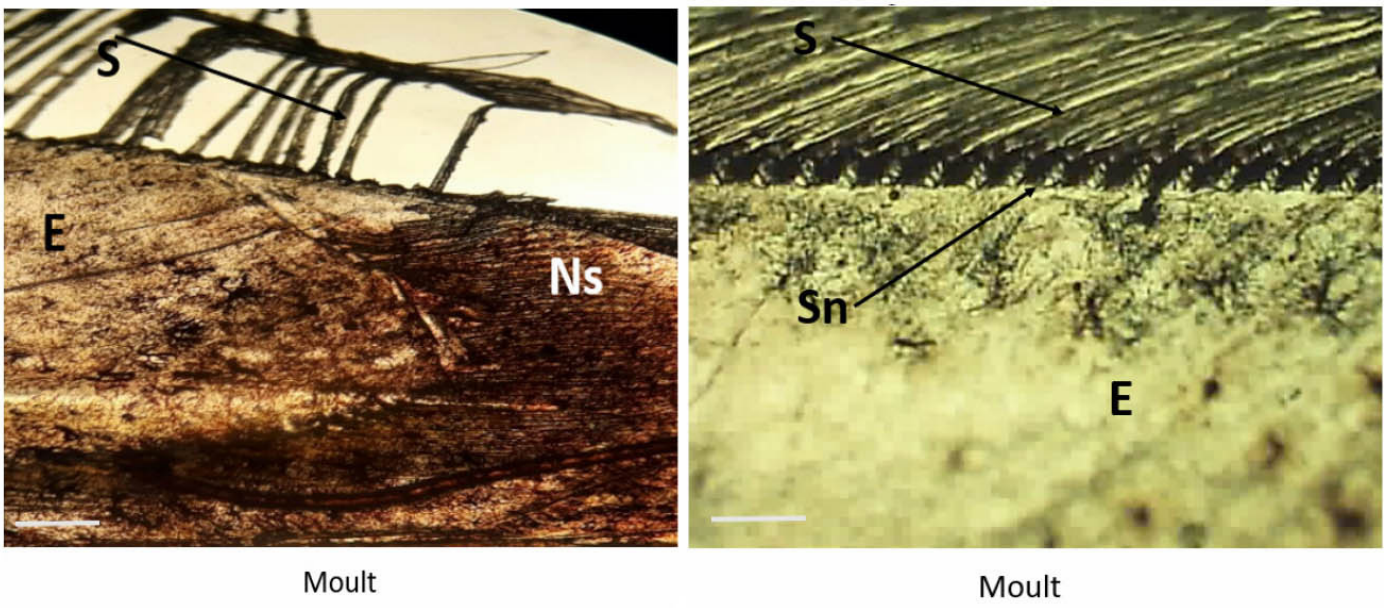

Figure 5 : Ventral view of uropod setae E stage (Magnification: 40 X)

Determination of moult stages. Uropods of the *Metapenaeus affinis* shrimps were examined and photographed under a light microscope (Olympus BX40) connected to a digital camera (DCE-2). The criteria used for moult staging followed Drach staging. Scale Bar =50 µm. EE=epidermal edge, L= wite layer, S= setae, SC= seta cone, Sn=setal node, Ns= new setae, E=epiderme.

= wavy edge of epidermis.

= clear zone between cuticle and epidermis.

Conclusion

The results of this study provide a basis for further study of the processes associated with the moulting cycle, such as complete separation of the external structure and dynamic exoskeleton formation, it is considered as an indicator of the development of shrimp, including continuous early development stages and the entire moulting cycle. It establishes a program for exoskeleton development, identification of the characteristics of the stages of ecdysis by setagenesis, and conclude the events involved from the several perspective, including exoskeleton formation, regulation, synthesis, degradation, mineral absorption /reabsorption, calcification and hardening, etc. As well as it is a simple, easy and practical means to assist aquaculture practitioners and researchers in determining the stages of individual moulting .These results will provide evidence for serve as a blueprint for other arthropods in future research.

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