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RESEARCH ARTICLE

Changes in uropod setae during molting of the freshwater crayfish, *Procambarus clarkii* (Cambaridae) from the River Nile, Egypt

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Abstract

Molting cycle is an essential process for crustacean growth, associated with several changes, so this study was carried out to investigate how telson setae and uropods are changing during different molt stages and substages of crayfish *Procambarus clarkii*. Microscopically, distinct changes in morphological structure and color pattern of the examined setae from different molting stages especially preecdysis (premolt sub stages) and post ecdysis (A-B) were exhibited. Bases of uropod setae, setal lumens, setal matrices, and setal cones showed remarkable changes during molting cycle from substages D1 (early premolt) to stage B (post ecdysis), with distinct variations in epidermal retraction, pigmentation and setal development.

INTRODUCTION

Molting cycle influences many aspects of crustacean biology, including animal morphology, cellular metabolism, physiology and behavior. Growth in crustaceans is not a continuous process, but takes place in successive steps during certain periods (Warner, 1977; Lowery, 1988, Hartnoll, 2000). Crustaceans must be first loose the connections between their living tissues and old cuticle, then they move out rapidly from the confines of this cuticle by taking up water to expand the new, flexible exoskeleton, and quickly harden it as described in details by Lowery (1988), so it provides protection and support for locomotion. The actual act of shedding the old exoskeleton, ecdysis, is the most obvious manifestation of the molt cycle. However, it comprises only a few minutes of a cycle that in some crustacean species takes from few days or week (in juveniles) to few months and even a year or more in adults of some species to complete. This cycle divides into several major stages with numerous substages according to Zilli *et al* (2003).

Using histological and morphological methods, Drach (1939) described in great detail molt-related anatomical changes occurring regularly in the integument of adult edible crabs, *Cancer pagurus.* Based on variations in the hardness of the cuticle as well as in epidermal and cuticular structures, he proposed for the molting cycle a classification system comprises five principal stages (A–E) and numerous substages. This system was later further elaborated by Skinner (1962) and Drach and Tchernigovtzeff (1967) in numerous studies on adult decapods as well as on other crustaceans (Charmantier-Daures and Vernet, 2004). Various criteria are used for staging the crustacean molt cycle, included the degree of hardness of the exoskeleton, changes in matrices of the setae (Drach, 1939), the growth of re-generating limb buds (Skinner, 1962; Stevenson *et al.*, 1968; Stevenson and Henry, 1971; Hopskin, 1982) and the progressive development of gastroliths in the digestive organs(Lowery 1988; Chang, 1995).

In addition to the anatomical and morphological changes, staging of molting cycle was also accompanied by other changes in behavior, physiology and biochemistry including cyclic activities of an antagonistic hormonal control (Skinner, 1985; Chang, 1995; Charmantier-Daures and Vernet, 2004). Hence, knowledge of the course of the molting cycle stage is highly important for the understanding of various aspects of crustacean biology, including physiology and biochemistry (Spindler-Barth, 1976; Chang, 1995; Ahearn *et al.*, 2004; Gaxiola *et al.*, 2005), behavior (Thompson and McLay, 2005; Mikami, 2005), food requirements (Mantelatto and Christofoletti, 2001;

Giménez *et al.,* 2002; Schmidt *et al.,* 2004), reproduction (Diaz *et al.,* 2003; Tarling and Cuzin-Roudy, 2003; de Lestang and Melville-Smith, 2006), accumulation dynamics of toxic substances (Bondgaard and Bjerregaard, 2005; Norum *et al.,* 2005), as well as fisheries and aquaculture of commercially important species (Ziegler *et al.,* 2004; de Oliveira *et al.,* 2006; Brylawski and Miller, 2006).

Therefore, this work aims at throw light on the morphological changes in uropod setae during molting to be used as criteria for molt staging in the crayfish, *Procambaraus clarkii*, and provides a simple and practical guide for the identification of the molting substages in this invasive species in Egypt.

Materials and methods

- **Sampling:**

Specimens of the freshwater crayfish, *Procambarus clarkii*, were collected alive for this study from the River Nile tributaries at Al-Kanater Al- Khairiya, Qalyoubiya Governorate, during the period from spring 2013 to autumn 2013. These specimens were transported to the laboratory in the Faculty of Science, Al-Azhar University, Nasr City, Cairo. All collected individuals were sexually immature. They were sexed and weighed to the nearest 0.1 gm using an electric balance with an accuracy of 0.01 g after blotting excess water with absorbent tissues. The total body length, standard length (length without telson and uropods), were measured with a Caliper Vernier with an accuracy of 0.01 mm. They were varied from 2.41 to 4.30 cm in standard length and from 0.27 to 2.09 g in total body weight.

- **Determination of morphological changes in uropod setae:**

Uropods of the red swamp crayfish were examined and photographed under a light microscope (Olympus BX40) connected to a digital camera (Echoo-Imager 502000). The criteria used for molt 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

The present results showed remarkable morphological changes in uropod setae during different molting stages and substages as following:

– **Intermolt (Stage C):**

This stage was characterized by complete formation of setal cone arranged in one row at the bases of setae, and without pigment retraction (Figure, 1). Epidermal tissue appear attached to setal cone and spreading between it. Retraction of setal matrix (Sm) was observed near the setal bases while setal lumens (Sl) were almost empty (Figure 1).

- Premolt (Stage D):

This stage was the longer molting stages and divides into four substages as following:

Substage D1:

Pigments are being retracted within epidermal layer at this substage. Setal lumens are clear without setal matrices (Figure, 2).

Substage D2:

This substage was characterized with the appearance of straight narrow white zone between epidermal tissue and setal cones indicating the formation of new cuticle. Pigments of epidermis are variable in appearance (Figure, 3).

Substage D3:

This substage is defined by the disappearance of setal cones, accompanied with more pigment retraction and initiation of new setae within each setal base. Pigments retracted from the bases of setal nodes and leave old cuticle. A wavy-shaped, dark edge appears between proximal border of epidermal tissues and bases of setae (Figure, 4).

Substage D4:

During this substage, intense pigment retraction from old cuticular setae was observed. A remarkable increasing in wavy- like edge at proximal border of epidermal tissue appears, associated with highly widened clear

zone between epidermal tissue and old setal bases (appearance of apolysis). New setae were clearly observed parallel to edge of epidermal tissue (Figure 5).

-An ecdysis (Stage E):

This stage was characterized appearance of new and protruded setae without setal cones. Setal lumens are being filled with setal matrix. Epidermal pigments are faint and less dense comparable with other previous subtsages (Figure, 6).

– Postmolt molt (Stages A & B):

In stage A (newly molt), new setae are clear, with less distict etale lumens, bases and nodes. Epiderms faint, with less dense pigments (Figure, 7 A).

On the other hand, at the onset of stage B, setal lumens, base and nodes become more distinct. Epidermal pigments are dense and darker. Setal lumens are filled with setal matrix. (Figure, 7 B).

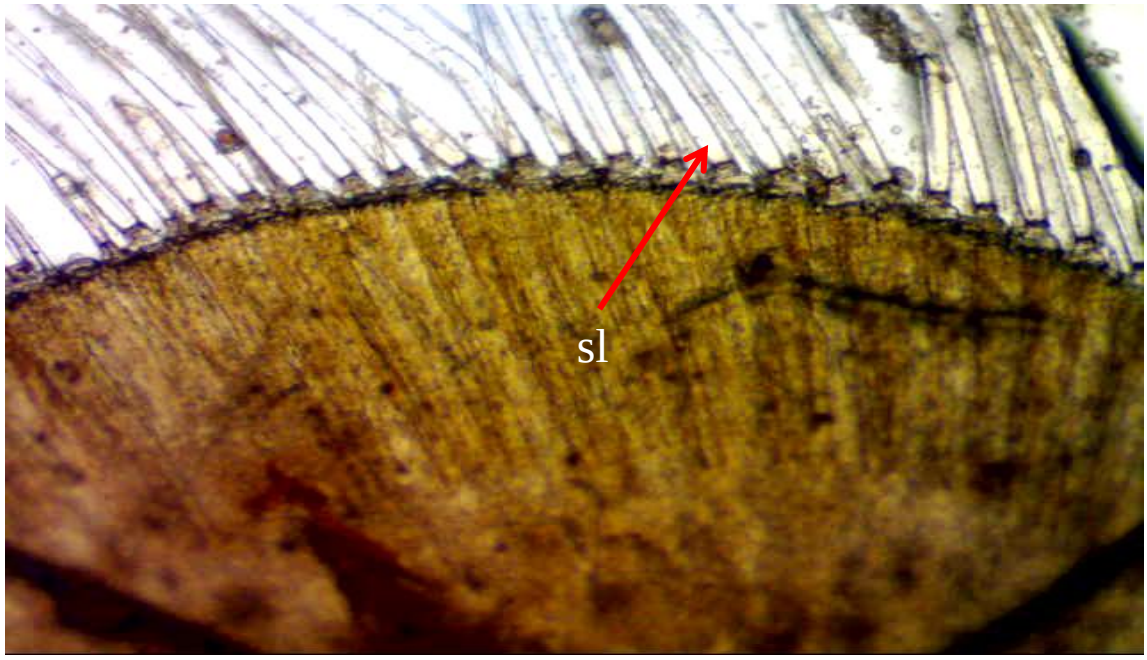
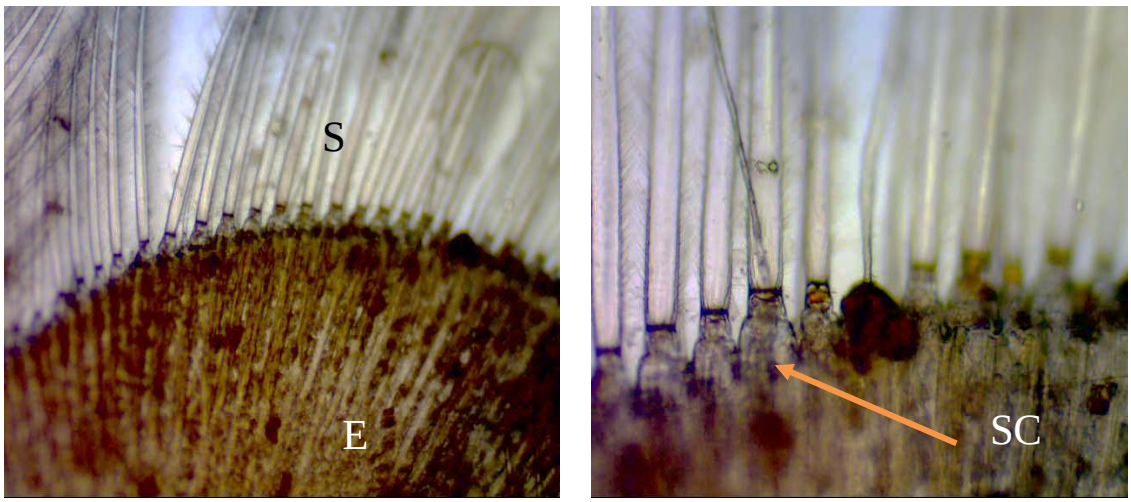
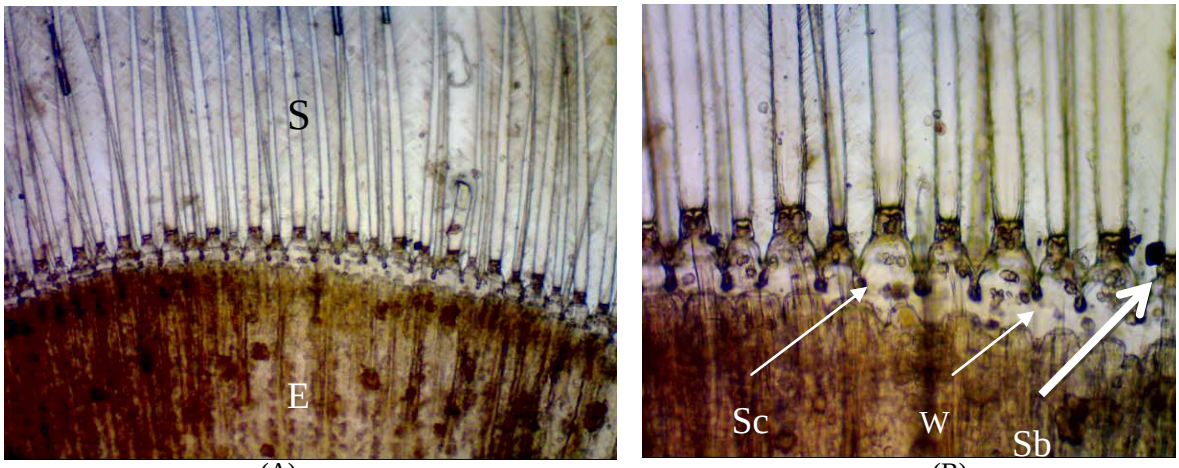


Figure 1: Ventral view of uropod setae stage C with magnification of 10X .



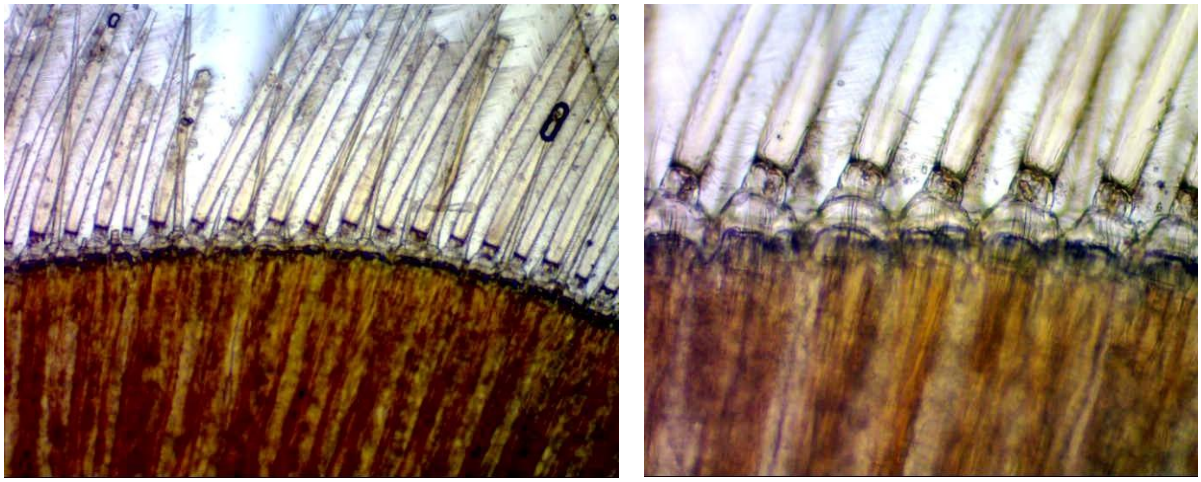
(A) (B)

Figure 2: Ventral view of uropod setae premolt stage D1-D2 with magnification of 4X (A), and 10X (B).



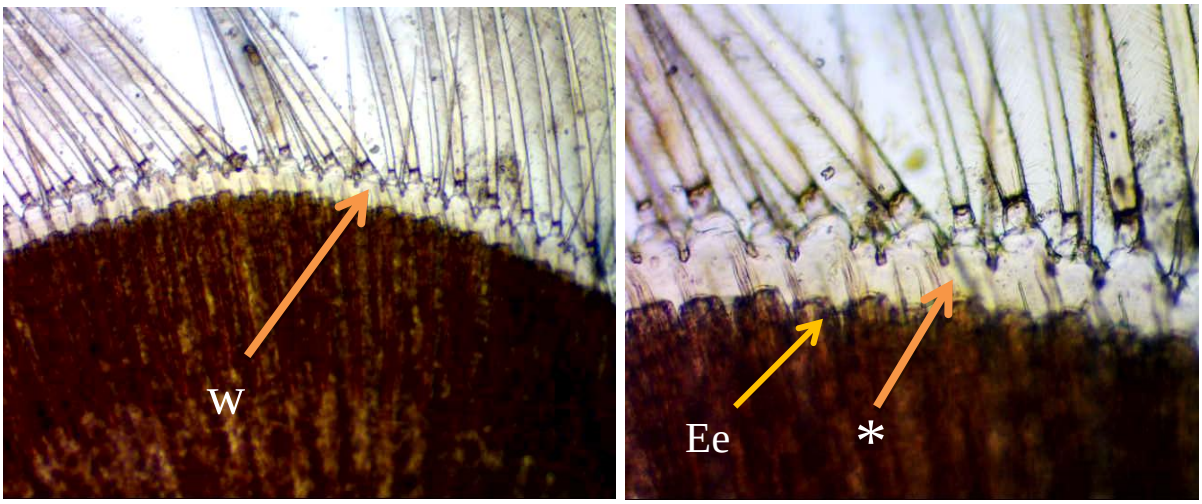
(A) (B)

Figure 3: Ventral view of uropod setae premolt stage D2 with magnification of 4X (A), and 10X (B).



(A) (B)

Figure 4: Ventral view of uropod setae premolt stage D3 with magnification of 4X (A), and 10X (B).



(A) (B)

Figure 5: Ventral view of uropod setae premolt stage D4 with magnification of 4X (A), and 10X (B).

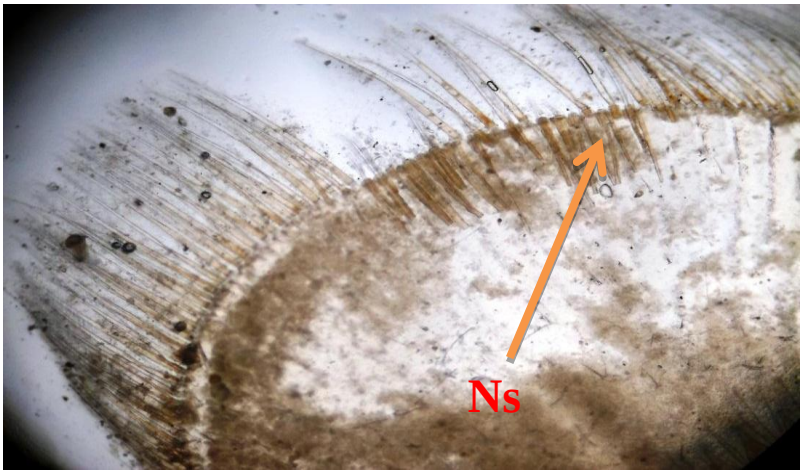
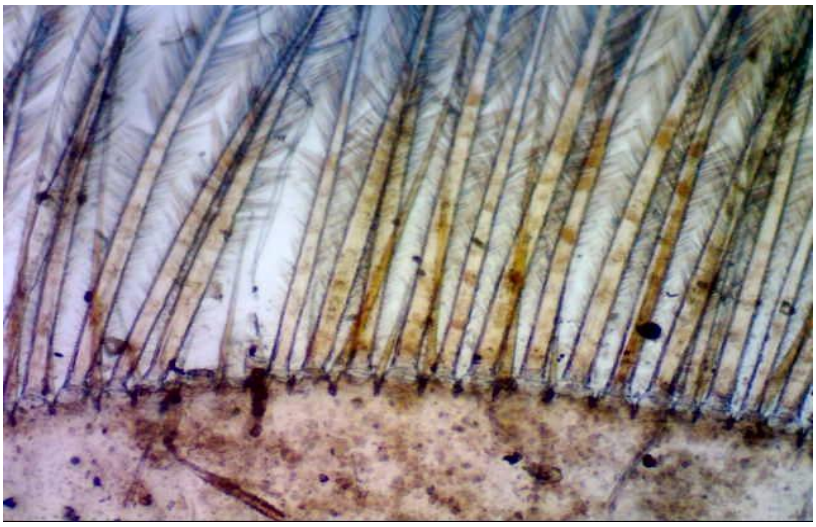
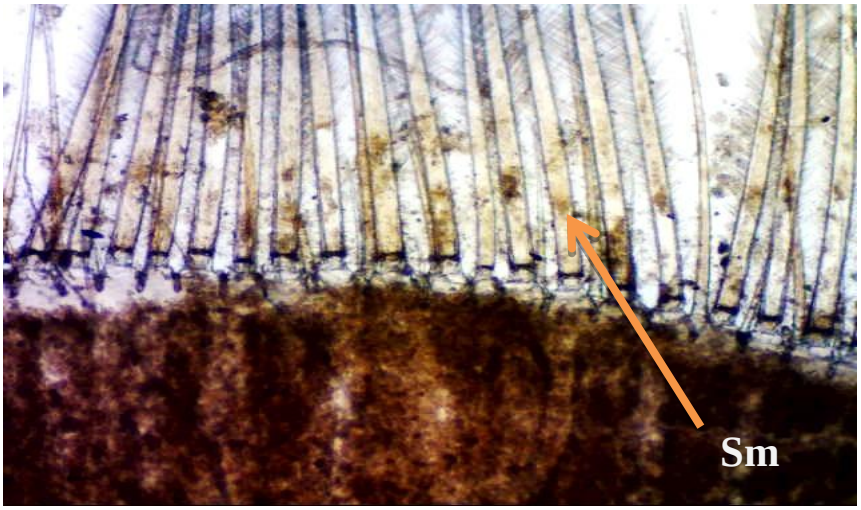


Figure 6 : Ventral view of uropod setae E stage (Magnification: 4 X).



(A)



(B)

Figure 7: Ventral view of uropod setae postmolt stage A- B with magnification of 10X (A), and 10X (B).

Discussion

Changes in integument reflect, more than other tissue, the periodic regulated growth that taking place in the crustaceans during molting (Yamaoka and Scheer, 1970). However, the lack of numerous precisely defined substages in researches including crustacean aquaculture, may not be a serious problem, as an identification of a few major molt stages should be sufficient to allow for a separation of fairly homogeneous materials taken from large cultures, e.g. for subsequent experiments, physiological measurements, or biochemical analyses. The present study may, therefore, provides a simple and practical guide for the identification of the molting substages in *Procambarus clarkii*.

Although setogenesis has been used as a criterion for molt 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 molt stages (Chan, *et al*., 1988).

The results of the present study showed distinct morphological changes in uropod setae of *P. clarkii* during molting cycle especially premolt substages (D_1-D_4). In the intermolt (Stage C), setal cone were arranged in one row at the bases of setae, without pigment retraction. Epidermal tissue appear attached to setal cone and spreading between it. These characters were similar to that described by Promwikorn, *et al.* (2004) on the black tiger shrimp (*Penaeus monodon*). While Zilli, *et al*. (2003), mentioned that the pigment seemed to form an epidermal line at the bases of the setal nodes of some shrimp species.

The present results showed that, during premolt substages (D_1-D_4) 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 (Drach, 1963; Skinner, 1985; Stevenson, 1985; Zilli, *et al*. 2003; Promwikorn, *et al.* 2004).

On the other hand, the setal lumens matrices varied in molting sub-stages from fulfilling in mete-ecdysis to complete retraction in premolt. These results are similar with that demonstrated by Chan, *et al*. (1988).

In spite of these results which can be used for determining molt stages in *P. clarkii*, further studies particularly physiological and chemical analyses are necessary to give complete information on the very complicated molt cycle of this species and other crustaceans used in aquaculture.

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Abbreviations: Ee = epidermal edge, S = setae, Sc = setal cone, Sm=setal matrix, Sl= setal lumens, Sb= Setal base, Ns = newly-formed seta, W= wavy edge of epidermis, * = clear zone between cuticle and epidermis.