

# STUDIES OF THE CLAWS OF THE CRAB *CYRTOGRAPSUS ANGULATUS* DANA (CRUSTACEA, BRACHYURA, GRAPSIDAE)

Fernández Gimenez, A.V.<sup>1</sup>  
Goldemberg, A.L.<sup>2</sup>  
Díaz, A.O.<sup>2</sup>

## ABSTRACT

The present work describes the histological characteristics and composition of the closer muscle fibers of *Cyrtograpsus angulatus* (Dana, 1851). Intermolt adult males were sampled in Mar Chiquita lagoon (Buenos Aires, Argentina). The claws were dissected and processed by standard histological and histochemical techniques. The composition of the fibers present in the muscle was analyzed measuring the sarcomere length at three different zones (dorsal, central and ventral). Muscular fibers showed evident striation, elongated nuclei with finely granular nuclear chromatin, and abundant loose connective tissue with amorphous substance, melanin, collagen fibers and haemocytes. The closer muscle presents different kind of fibers, being predominant the slower fibers. The relationship between the structure and function of the muscle and the possible correspondence between the mechanical power produced by the closer muscle and the sarcomere length of the fibers is under discussion.

**Keywords:** Brachyura, claw, crab, Crustacea, histology, sarcomere.

## CARACTERIZAÇÃO DO MÚSCULO ADUTOR DA QUELA DE *CYRTOGRAPSUS ANGULATUS* (CRUSTACEA, BRACHYURA, GRAPSIDAE)

### RESUMO

O presente trabalho descreve as características histológicas e a composição das fibras do músculo de fechamento da queela de *Cyrtograpsus angulatus* (Dana, 1851). Foram estudados machos adultos em intermuda provindos da laguna de Mar Chiquita (Buenos Aires, Argentina). Cada queela foi separada, dissecada e processada com técnicas histológicas e histoquímicas padrão. A composição das fibras presentes no músculo foi analisada medindo o comprimento do sarcômero em três zonas do músculo (dorsal, central e ventral). Foram observada fibras musculares com estriação evidente e núcleos alongados com cromatina frouca, abundante tecido conectivo frouco com substância amorfa, melanina, fibras colágenas e hemocitos. Foi determinado que o músculo de fechamento apresenta diferentes tipos de fibras, com o predomínio das fibras lentas. É fator de discussão a relação entre estrutura e função do músculo e a provável correspondência entre a força mecânica produzida e o comprimento dos sarcômeros das fibras.

**Palavras-Chave:** Brachyura, queela, caranguejo, Crustacea, histologia, sarcômero

### INTRODUCTION

Arthropod muscles have morphological characteristics similar to those of the striated muscles of the vertebrates; however morphological, physiological

<sup>1</sup> CONICET (Consejo Nacional de Investigaciones Científicas y Tecnológicas).

<sup>2</sup> Departamento de Biología. Facultad de Ciencias Exactas y Naturales. Universidad Nacional de Mar del Plata. Funes 3250. Mar del Plata, Argentina. E-mail fgimenez@mdp.edu.ar.

and ultrastructural differences exist among the different groups of arthropods and also among the crustaceans (ROYUELA et al., 1998).

Crustacean muscles come in a wide variety of different types and show amazing functional adaptations. Crustacean muscle fibers show great diversity in their morphological and physiological features (ATWOOD, 1973). The physiological properties are correlated with ultrastructural characteristics. Muscular fibers with short sarcomeres usually present fast contraction and little isometric tension, meanwhile fibers with long sarcomeres contract slowly but produce large tensions. In addition to this there is also a large spectrum of fibers with intermediate characteristics (MELLÓN, 1992).

In brachyuran crustaceans only the first pair of pereopods has claws and plays functions related with feeding, locomotion and reproduction. The claw presents two muscles: a dorsal opener muscle and a ventral closer muscle (LANG et al., 1977 a; b). In several crustacean species was demonstrated that the closer muscle is more important due to its volume and the production of important mechanical power (TAYLOR, 2000). Considerable literature exists on closer muscle in relation to the distribution of muscle fibers (LANG et al., 1977a; b; STEPHENS et al., 1984; GOVIND; BLUNDON, 1985; CLAXTON et al., 1994; LONGO et al., 2005). However basic information on the histological characteristics of the closer muscle in decapoda is not available. In particular, reports can be found in the literature on the *C. angulatus* closer muscle. The present work introduces for the first time histological studies and examines the fiber composition of the closer muscle of *C. angulatus* (Dana, 1851). This specie is distributed along the Atlantic Ocean from Rio de Janeiro (Brazil) to Puerto Deseado (Argentina) (BOSCHI, 1964) and along the Pacific Ocean from San Lorenzo Island (Perú) to Talcahuano (Chile) (RETAMAL, 1981). This species is found in dense populations in marine environments, estuaries and coastal lagoons (SPIVAK et al., 2001).

## MATERIALS AND METHODS

Adult males were obtained from Mar Chiquita lagoon. Crabs were immediately transferred to the laboratory in cubes with water from the collection place. Then, they were acclimated for ten days in aquarium with filter and continuous aeration. The experimental conditions were: temperature  $22 \pm 2$  °C, salinity 35ups, and photoperiod 12:12 (L: D). They were fed with a commercial feed (Cichlind T.E.N., Wardley, USA) three times a week (0.07 g per individual). Before taking the samples, they were starved for 48 hours. The molt stage was determined by the observation of the setae from the maxilla (MORIYASU; MALLETT, 1986) and intermolt individuals were selected for the posterior study.

## HISTOLOGICAL AND HISTOCHEMICAL CHARACTERIZATION

Individuals were anesthetized on ice for 30 minutes and then the chelipeds were cut. The claws were separated and aqueous Bouin was injected into the muscular mass. Then, the muscular mass was fixed in the same fluid for 48 hours and stored in alcohol 70%. The tissue was dehydrated in graded series of ethanol, and then it was placed in butyl alcohol, butyl paraffin (50: 50 v/v) at 55°C for 24 hours, and finally embedded in paraffin. Sections (5 µm) were cut and stained with standard and histochemical techniques (Table 1).

The fibers width in the closer muscle was measured on histological sections (haematoxylin-eosin) from three individuals using ocular micrometer. Five fields were analyzed in each section measuring five fibers / field.

## MUSCLE COMPOSITION

The morphological observation of the sarcomere length was used to characterize the type of fibers present in the closer muscle (LANG et al., 1977a). For this purpose four intermolt adult males of  $3.55 \pm 0.220$  cm carapace width were examined. They were anaesthetized on ice for 30 minutes and then the chelipeds were cut. The muscular tissue was fixed “*in situ*”, in a relax state by holding the dactyl open while Bouin’s fluid was injected through the exoskeleton. Then, the integument was cut in several places to facilitate the penetration of the fixative into the muscular mass, and both claws were immersed in the same fluid for 48 hours. After the fixation period, the closer muscles of both claws were removed and stored in alcohol 70 %.

Each closer muscle was divided into three zones: dorsal, central and ventral, taking four samples of muscular fibers from each zone. Three series of six consecutive sarcomeres and three series of three A-bands were measured in each sample. The measurements of the sarcomere length (n=216) and A-band (n=108) were made using an ocular micrometer (Carlzeiss Jena NU2). The results were statistically analyzed with the Student-t test, ANOVA, the Kolmogorov-Smirnov’s test for two series of data, and lineal regression analysis (SOKAL; ROLFH, 1979).

## RESULTS

### HISTOLOGICAL CHARACTERIZATION

In the propus, the opener muscle is in dorsal position and the ventral closer muscle is larger. Both muscles are separated by connective tissue with abundant parallel collagen fibers (fig.1a). The integument consists of a cuticle composed by proteins and quitin and is secreted by the underlying epidermis (STEVENSON, 1985). The epithelium is columnar and single layered. The nucleus and vacuoles are located in the basal and apical region of cells, respectively. Underlying the epithelium it was observed a loose connective tissue with

abundant fibroblasts, haemocytes, collagen fibers and melanin, in addition to the extracellular matrix.

The apodeme of the closer muscle has similar histological characteristics to those of the integument, particularly fibrous connective tissue rich in collagen fibers and melanin; the muscular insertions are clearly evident.

The closer muscle presents well-defined muscular fibers with conspicuous striation forming fascicles. Each fiber has sarcomeres that evidence A and I bands, and also the Z-line (fig. 1b).

The muscular fibers contain numerous elongated nuclei along the fibers that are located peripherally under the cellular membrane; the cytoplasm is acidophilic with a clearer zone around the nucleus (fig. 1c).

Each muscular fiber is surrounded by a capsule of reticular and elastic fibers, that along together with the glycosaminoglycans contribute to the formation of an external PAS-positive layer.

The same technique evidences the presence of glycogen with a heterogeneous arrangement. The muscular fibers width varied between 7.82 and 8.94  $\mu\text{m}$  (Table 2).

The connective tissue surrounds the individual fibers and also the groups of fibers forming fascicles. In the connective tissue there are vessels and haemolymph sinuses generally associated with nerves.

The connective tissue comprises cells and extracellular matrix composed by fundamental substance and fibers.

Different cell types are observed; some of them are exclusive of the connective tissue, whereas other belong to the immune system. The typical connective cells are fibroblasts that can be found active or inactive (fibrocytes). Fibroblasts are elongated cells with pale cytoplasm. The most evident feature of these cells is the high basophilic nucleus, ovoid and granular with conspicuous nucleoli. The fibrocytes are smaller with acidophilic cytoplasm. Their nucleus is smaller, elongated and more basophilic.

The cellular elements of the immune system include hyaline haemocytes and granulocytes of two types, semi-granular and granular (fig. 1d). The hyaline haemocytes are nearly circular with scarce cytoplasm and a large central nucleus containing loose chromatin. Granulocytes are bigger than hyaline haemocytes, and both semi-granular and granular have acidophilic cytoplasm with PAS positive granules. The semi-granular granulocytes evidence a central nucleus, while the granular ones have an eccentric nucleus.

The connective tissue presents acidophilic extracellular matrix. The proteoglycans or mucopolysaccharides and in particular the glycosaminoglycans are responsible for the affinity of the fundamental substance with PAS and Alcian Blue techniques. Collagen, reticular and elastic fibers are present in the extracellular matrix.

## MUSCLE COMPOSITION

The sarcomere length range (SL) varies between the right and left claw. The population of muscular fibers of both claws was compared with the Kolmogorov-Smirnoff test for two samples of data, showing significant differences in all the individuals (Table 3).

In all cases, a similar pattern of distribution of fibers is observed. At the dorsal and ventral zones, the SL varies between 8 and 14  $\mu\text{m}$ , evidencing only the presence of slow fibers, while at the central zone it varies between 1.6 and 12  $\mu\text{m}$ , being the slow fibers the most frequent (Figure 2).

The percentage composition of the type of fibers according to the SL for each individual is shown in table 4. SL values vary significantly among the different zones of the muscle in the right claw of all the individuals. However, in the left claw the SL of the central zone is statistically different to that of the dorsal and ventral zones.

In all the individuals the SL and the A-band length show a lineal regression with  $R^2$  values between 0.5923 and 0.7584.

## DISCUSSION AND CONCLUSION

The crustacean muscular system plays an important role in the interaction between the organisms and the environment. Each species presents a characteristic neuromuscular pattern that varies in relation to its feeding, reproductive and defense habits.

*Cyrtograpsus angulatus* claws have two muscles, the opener in dorsal position and the closer in ventral position. This characteristic was previously described for other species such as *Homarus americanus* Milne Edwards, 1837 (LANG et al., 1977a; b), *Callinectes sapidus* Rathbun, 1896 (GOVIND; BLUNDON, 1985), *Uca pugnax* Smith, 1870 and *Gecarcinus lateralis* (Freminville, 1835) (MYKLES, 1988). The opener and closer muscles represent one of the main muscular masses of decapod crustaceans (EL HAJ; WHITELEY, 1997).

In *C. angulatus* closer muscle, as in other decapod (JOHNSON, 1980), the A- and I-bands, and Z-line are observed forming the sarcomere.

In several crustacean species was proved that the presence of fibrillar components in the connective tissue associated with muscular insertions of the integument is related to the transmission of strength from the muscle to the exoskeleton (MELLÓN, 1992). The tension generated by muscles to produce movement is transmitted to the exoskeleton directly or through articular tendons called apodemes. In the present study, the individual muscular fibers are joined to the epidermic cells of the integument by one end and to the apodeme by the other. In *C. angulatus*, the muscular fibers of the closer muscle are arranged forming an angle in relation to their insertion in the apodeme. This arrangement was also observed in *Chasmagnathus granulatus* and is related to a major efficiency in crushing and cutting food (BOND-BUCKUP et al., 1991).

The present work demonstrates that the location of glycogen in the muscular fibers varies considerably inside the same section. This aspect was also observed by JOHNSON (1980) in the blue crab *C. sapidus*. Regarding to the molting cycle, the secretion of the new cuticle involves enormous energy consumption, and part of this process occurs while the animal is not feeding (before and after the ecdysis). For this reason, it is not surprising the presence of reserves such as glycogen in muscular tissues of crustaceans, and also in the hepatopancreas, epidermis, and subepithelial connective tissue during intermolt and early premolt (STEVENSON, 1985).

Abundant connective tissue is observed associated with the muscular mass in *C. angulatus*' claws. In general, the connective tissue comprises cells and fibers embedded in the amorphous extracellular matrix. Cellular elements include fibroblasts and haemocytes associated with haemal sinuses. Crustacean immune system is cellular and humoral. Haemocytes are circulating cells that take part in immune reactions (SMITH; SÖDERHÄLL, 1983; KONDO et al., 1998; RENDON; BALCÁZAR, 2003). In this work, acid mucopolysaccharides are evidenced by abundant extracellular matrix acidophilic and strongly positive with Alcian Blue. In the hepatopancreas of the freshwater prawn *Caridina sp.* Milne Edwards, 1837, Miyawaki et al. (1985) observed PAS positive extracellular matrix in the connective tissue, suggesting the presence of glycosaminoglycans and proteoglycans.

Several researchers have considered the collagen fibers as one of the most abundant fibrous component of the connective tissue in crustaceans (KOOKA-CISCO; TALBOT, 1986; FARRELY; GREENAWAY, 1987). The elastic fibers constitute an additional fibrous component; in *C. angulatus* they were evidenced by the Spirit Blue technique (ELDER, 1973) around the muscular fibers and in the extracellular matrix. Differences have been observed in the elastic fibers from both vertebrates and invertebrates. Elastin is not present in the second one (ELDER; OWEN, 1967; ELDER, 1973).

In the present study the fibers diameter in the closer muscle varies between 7.82 and 8.94  $\mu\text{m}$ . In general, the diameter of the muscular fibers is very variable among crustaceans. Diameters vary from 10  $\mu\text{m}$  in skeletal muscular fibers to 5 mm in the depressor muscle of the barnacle *Balanus nubilis* Darwin, 1854. In the crayfish, BITTNER (1989) found that the opener muscle has fibers from 40 to 500  $\mu\text{m}$  diameter. Small diameter fibers tend to produce slow contractions, though muscular fibers with similar diameters from different muscles can show different speeds of contraction (HOYLE; SMYTH, 1963).

In contrast, several authors showed a positive correlation between the sarcomere length and the speed of contraction. Fibers with short sarcomeres (2-4  $\mu\text{m}$ ) are generally fast and produce less force per unit of area than fibers with long sarcomeres (> 6  $\mu\text{m}$ ) (ATWOOD, 1973; WARNER et al., 1982; GOVIND; BLUNDON, 1985; TAYLOR, 2000). Slow fibers show a specific structure related to their physiological role, which not only

includes long sarcomeres, but also a high relation between thin filaments and thick filaments. Measuring the sarcomere length in *C. angulatus*, different types of fibers are observed in the closer muscle, being the slow fibers predominant. Intermediate and fast fibers are only present at the central region of the muscle. This feature is also found in other crab of the same family *C. granulatus* that co-inhabit in Mar Chiquita lagoon; however, in this species fast fibers are more abundant at the ventral region (LONGO et al., 2005).

In *C. angulatus*, the sarcomere length and A-band show a positive correlation; this characteristic was also observed in the closer muscle of six species of the genus *Cancer* Linnaeus, 1758 (TAYLOR, 2000).

An examination of the habits of the decapods may also reveal factors important in the evolution of cheliped size and structure (LEE, 1995). Claws are high-performance structures producing some of the strongest forces exerted by any animal, for a given body weight (TAYLOR, 2000). Communication in crustaceans often involves the display of antennae and chelipeds. The roles of the chelipeds in agonistic and aggressive interactions during inter and intraspecific competition for a limited resource is well documented in the literature (MARIAPPAN et al., 2000).

Studies about diversity of decapod claws emphasized the adaptative aspects of these structures. Big sized claws in crabs and lobsters are appropriate to manage and crush their preys' exoskeleton (TAYLOR, 2001).

The crab *C. sapidus* presents structural and functional asymmetry between the claws (crusher claw and cutter claw), which does not correspond to an asymmetry in the fiber composition of the muscle. The force generated by the crusher claw is significantly higher than that produced by the cutter claw; however both claws are entirely composed by slow fibers with long sarcomeres (GOVIND; BLUNDON, 1985). On the other hand, the lobster *H. americanus* exhibits a correspondence between the functional asymmetry and the type of fibers present in the muscle, the cutter claw comprises between 60 and 80% of fast fibers, and meanwhile the crusher claw is only composed by slow fibers (LANG et al., 1977 a; b).

Although *C. angulatus* is considered a littoral marine species, it is largely distributed in brackish water estuaries. Adults commonly inhabit on soft and muddy bottoms characterized by polychaete populations from the genus *Heteromastus* Eisig, 1887 and *Laeonereis* Hartman, 1945. This species of crab is omnivorous; it eats mainly annelids and carrion (OLIVIER et al., 1972). It is not surprising that the closer muscle structure, mainly composed by slow fibers, is related to the feeding habits of this species.

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Table 1. Histological and histochemical technique.

| Procedures               | Interpretation of staining reactions | References                     |
|--------------------------|--------------------------------------|--------------------------------|
| Haematoxylin and eosin   |                                      | MARTOJA; MARTOJA-PIERSON, 1970 |
| Masson's Trichrome Stain | Connective tissue.                   | MARTOJA; MARTOJA-PIERSON, 1970 |
| Mallory's Triple Stain   | Connective tissue.                   | MARTOJA; MARTOJA-PIERSON, 1970 |
| Reticulum stain          | Reticulin                            | MARTOJA; MARTOJA-PIERSON, 1970 |
| Spirit Blue              | Elastic fibers                       | ELDER; OWEN, 1967              |
| Scarba Red               | Nuclear stain                        | HUMASON, 1962                  |
|                          |                                      |                                |

|                                |  |                   |
|--------------------------------|--|-------------------|
| AB pH 2,8                      | Acid mucopolysaccharides                           | LEV; SPICER, 1964 |
| PAS                            | GPs with oxidizable vecinal diols and /or glycogen | MC MANUS, 1948    |
| Feulgen with picro-methyl blue | Granulocytes                                       | FARLEY, 1969      |

AB, Alcian Blue; PAS, periodic acid Schiff; GPs, glycoproteins

Table 2. Fibers width of closer muscle of *Cyrtograpsus angulatus*.

| Animal | Fibers width ( $\mu\text{m}$ )<br>$\bar{x} \pm \text{SD}$ |
|--------|---|
| 1      | 7.82 $\pm$ 1.278  |
| 2      | 8.55 $\pm$ 0.677  |
| 3      | 8.94 $\pm$ 1.019  |

Values are mean  $\pm$  standard deviation  $\bar{x} \pm \text{SD}$

Table 3. Sarcomere length in claw closer muscles of *Cyrtograpsus angulatus*.

| Animal (number) | claw  | Sarcomere length ( $\mu\text{m}$ ) |          | Claw length (mm) |
|-----------------|-------|------------------------------------|----------|------------------|
|                 |       | $\bar{x} \pm \text{SD}$            | range    |                  |
| 1               | right | 10.3 $\pm$ 2.28 <sup>a</sup>       | 2.4-14   | 210              |
|                 | left  | 10.5 $\pm$ 1.11 <sup>b</sup>       | 8-12     | 220              |
| 2               | right | 11.2 $\pm$ 1.94 <sup>a</sup>       | 8-14     | 173              |
|                 | left  | 10.0 $\pm$ 3.13 <sup>b</sup>       | 1.6-14.4 | 185              |
| 3               | right | 11.8 $\pm$ 1.98 <sup>a</sup>       | 6-14     | 184              |
|                 | left  | 11.6 $\pm$ 1.85 <sup>b</sup>       | 8-16     | 164              |
| 4               | right | 11.1 $\pm$ 1.69 <sup>a</sup>       | 8-14     | 185              |
|                 | left  | 10.6 $\pm$ 2.67 <sup>b</sup>       | 2.4-14   | 187              |

Values are mean  $\pm$  standard deviation  $\bar{x} \pm \text{SD}$

Values with different superscripts are significantly different ( $P < 0.05$ )

Table 4. Percentage composition of muscle fiber types in the paired claw closer muscles of *Cyrtograpsus angulatus*.

| Animal<br>(number) | Muscle<br>sections | Sarcomere length ( $\mu\text{m}$ ) |                     |            |             |                     |            |
|--------------------|--------------------|------------------------------------|---------------------|------------|-------------|---------------------|------------|
|                    |                    | Left claw                          |                     |            | Right claw  |                     |            |
|                    |                    | Short<br><4                        | Intermediate<br>4-6 | Long<br>>6 | Short<br><4 | Intermediate<br>4-6 | Long<br>>6 |
| 1                  | dorsal             | 0                                  | 0                   | 100        | 0           | 0                   | 100        |
|                    | central            | 0                                  | 0                   | 100        | 16.7        | 2.8                 | 80.4       |
|                    | ventral            | 0                                  | 0                   | 100        | 0           | 0                   | 100        |
|                    |                    |                                    |                     |            |             |                     |            |
| 2                  | dorsal             | 0                                  | 0                   | 100        | 0           | 0                   | 100        |
|                    | central            | 17                                 | 43                  | 40         | 0           | 0                   | 100        |
|                    | ventral            | 0                                  | 7                   | 93         | 0           | 0                   | 100        |
|                    |                    |                                    |                     |            |             |                     |            |
| 3                  | dorsal             | 0                                  | 0                   | 100        | 0           | 0                   | 100        |
|                    | central            | 0                                  | 0                   | 100        | 0           | 27                  | 73         |
|                    | ventral            | 0                                  | 0                   | 100        | 0           | 0                   | 100        |
|                    |                    |                                    |                     |            |             |                     |            |
| 4                  | dorsal             | 0                                  | 0                   | 100        | 0           | 0                   | 100        |
|                    | central            | 17                                 | 6                   | 77         | 0           | 0                   | 100        |
|                    | ventral            | 0                                  | 0                   | 100        | 0           | 0                   | 100        |



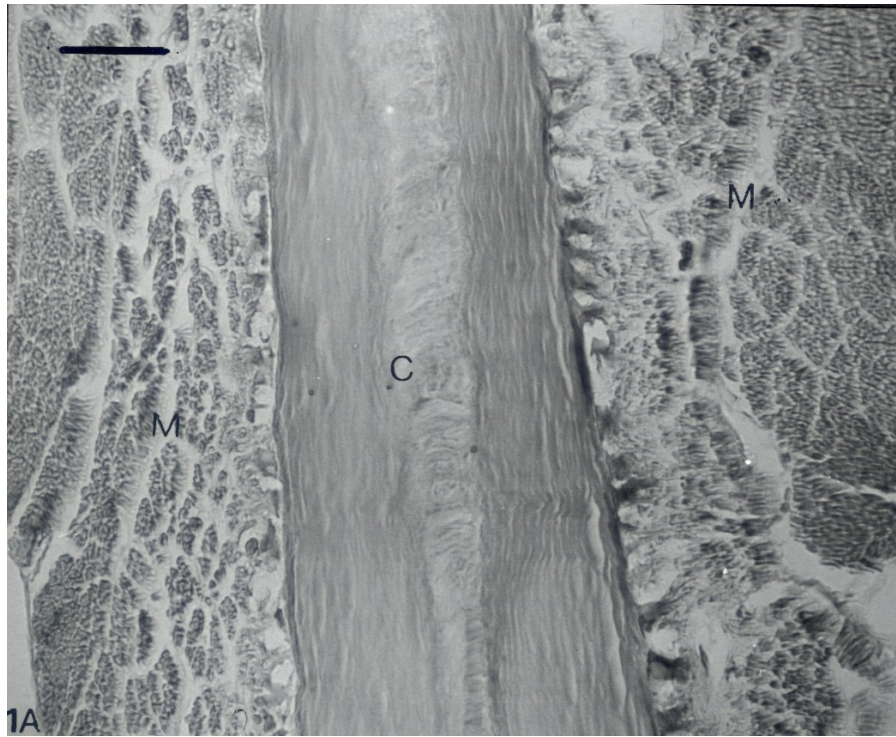


Figure 1a. Portion of the propus from *Cyrtograpsus angulatus*. Note the transversal muscle section (m) separated by connective tissue with collagen fibers (c). (Mallory's Triple Stain). (Bar: 30 $\mu$ m)

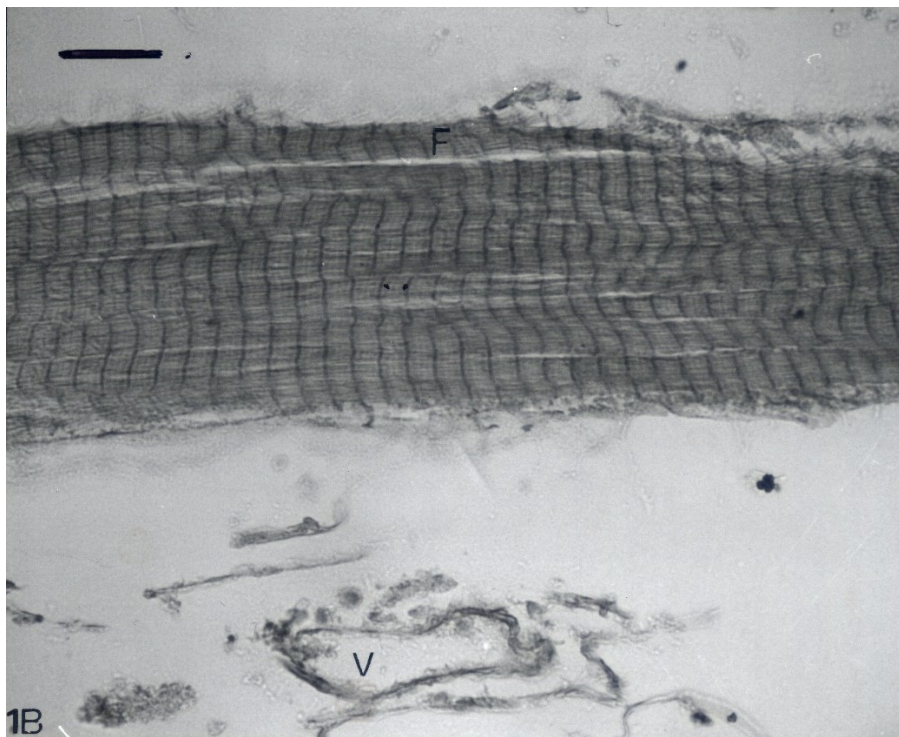


Figure 1b. Longitudinal section of closer muscle from *Cyrtograpsus angulatus*. Note the A (dark) and I (light) bands. The Z line is visible within the light. f: fiber, v: vessel (PAS staining). (Bar: 30 $\mu$ m)

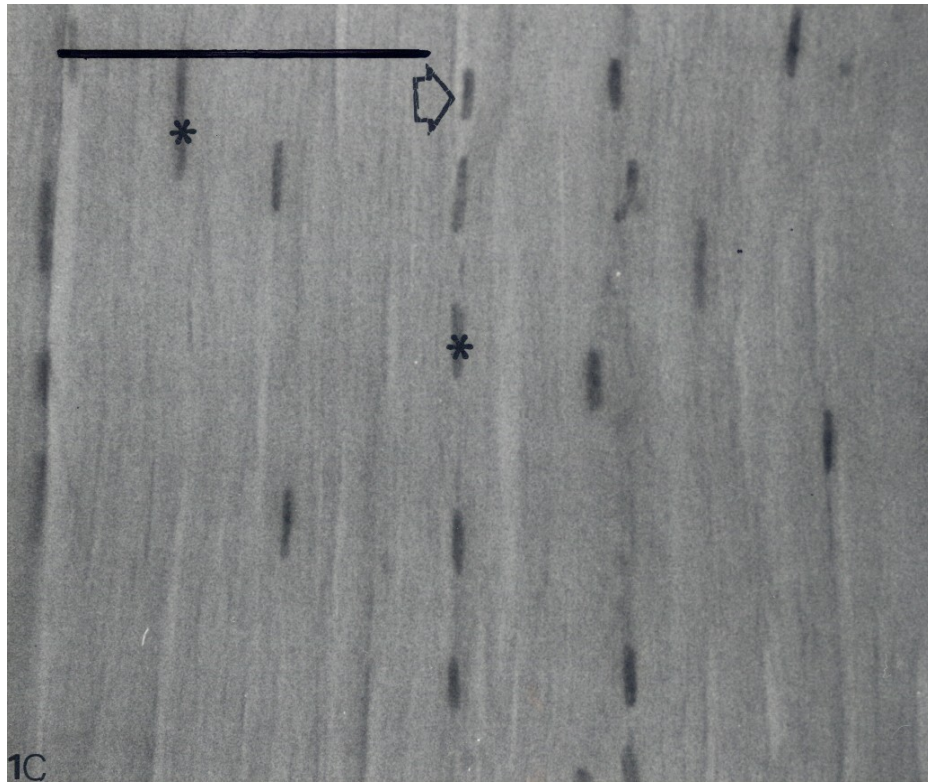


Figure 1c. Longitudinal section of closer muscle from *Cyrtograpsus angulatus*. The elongated nuclei (asterisk) are surrounded by a clear cytoplasmic zone (arrow). (Scarba Red). (Bar: 30µm)

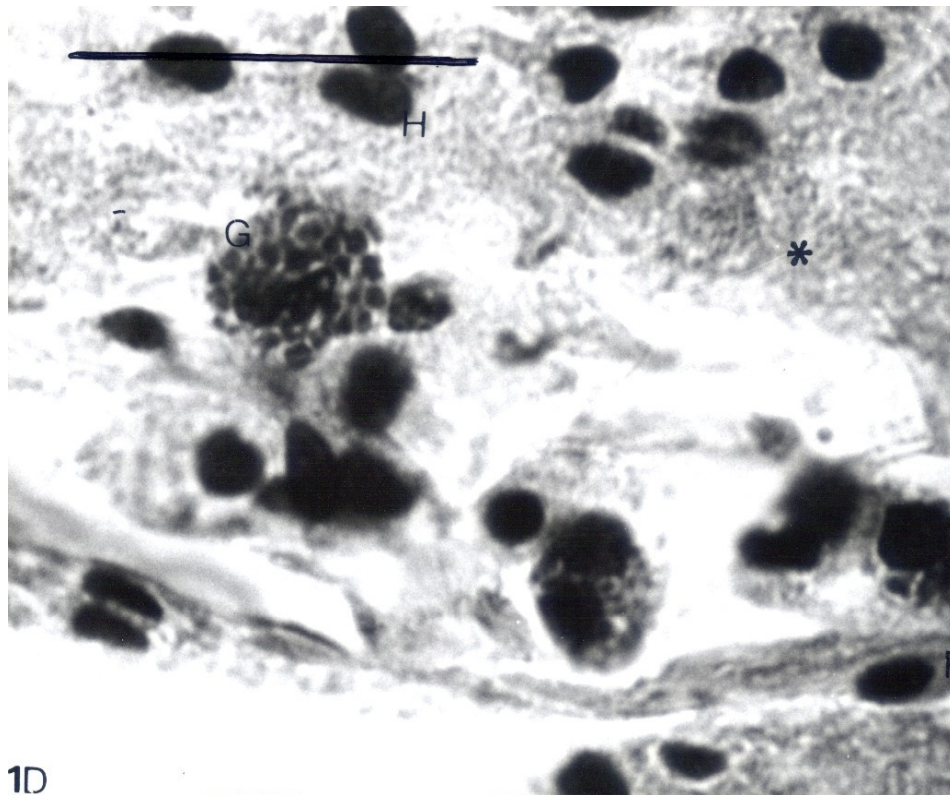


Figure 1d. Connective tissue associated to closer muscle of *Cyrtograpsus angulatus*, showing cells and extracellular matrix (asterisk). f: fibrocyte; g: granulocyte; h: hyaline haemocytes; (Fueigen with picro-methyl blue). (Bar: 30µm)

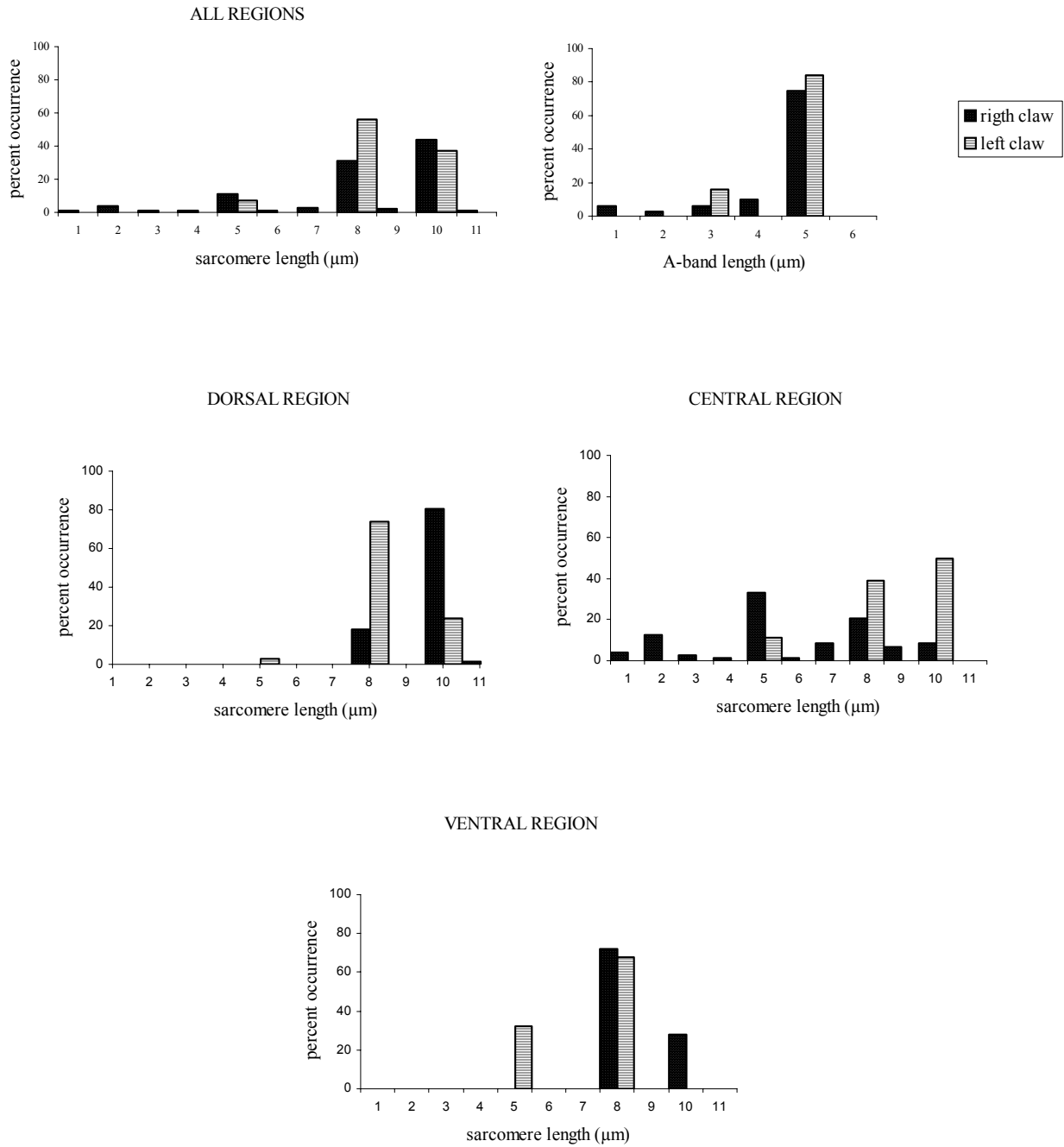


Figure 2. Frequency histogram of fibers with characteristic sarcomere lengths and A-band lengths in the paired claw closer muscle of animal number 1 (carapace width = 3.62 cm).