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The claw closer muscle of *Neohelice granulata* (Grapsoidea, Varunidae): a morphological and histochemical study

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Abstract

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The claw closer muscle of *Neohelice granulata* was studied according to histological, histochemical, and morphometrical criteria. Adult male crabs in intermoult stage were collected from Mar Chiquita Lagoon (Buenos Aires, Argentina). Muscle fibers show evident striations and oval-elongated nuclei with loose chromatin. The loose connective tissue among muscle fibers consists of cells and fibers embedded in an amorphous substance. Muscle histochemistry reveals two slow fiber types: 'A' and 'B'. Prevailing A fibers are larger, and they usually show, with respect to B type, a weaker reaction to whole techniques. Fibers with short (SS), intermediate (IS), and long sarcomeres (LS) appear in the claw closer muscle, being the LS fibers predominant. Concluding, the histochemical and morphometrical characteristics of the claw closer muscle fibers of *N. granulata* are indicative of slow fibers. The slow A type (low resistant to fatigue) prevails.

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Introduction

The muscle tissue represents a major proportion of the total tissue mass in decapod crustaceans. It includes the deep and superficial abdominal flexor and extensor muscles, the claw closer and opener muscles, and the extensor and flexor muscles of the walking legs (El Haj and Whiteley 1997).

All crustacean muscle fibers are striated (Mellon 1992) and show a great diversity of morphological and physiological features. One of the most evident sources of morphological variation is the sarcomere length (Atwood 1973). The fibers present short (<4 μm), long (>6 μm) and intermediate values (4–6 μm) (Lang *et al.* 1977a,b; Govind and Lang 1978). Short sarcomere fibers produce fast, weak, and brief contractions, whereas long sarcomere fibers generate slow, powerful, and prolonged contractions (Atwood 1973; Govind 1984; Mellon 1992; Claxton *et al.* 1994; Taylor 2000).

In addition to morphological analyses of muscle fibers, histochemical methods for discriminating between the different types of fibers are also available. They involve tests of myosin-adenosine triphosphatase (m-ATPase) and oxidative enzymes activity (Neil *et al.* 1993). In the m-ATPase method, both preincubations at acid or alkaline pH and changes in the incubation temperature are useful to detect a greater variety of muscle fiber types (Mykles 1988; Günzel *et al.* 1993; Neil *et al.* 1993). The contraction speed and the oxidative capacity of the muscle fibers tend to vary inversely. Moreover, variations in m-ATPase activity have been related to differences in polysaccharide contents (Tse *et al.* 1983).

The claw is a multifunctional organ used during feeding, mating, agonistic interactions, and burrowing (Lee 1995). There are two muscles in the claw: the closer and the opener, which are involved in movements of the dactylus respect to the propodus. The closer muscle occupies a large proportion of the claw volume, producing some of the strongest mechanical forces reported for any group of animals (Taylor 2000).

The burrowing crab *Neohelice granulata* (Grapsoidea, Varunidae) is found in the benthic communities of estuaries and brackish coastal lagoons in the southwestern Atlantic region (Luppi *et al.* 2001). As one of the most frequent crabs in Mar Chiquita Lagoon, it inhabits mudflats and *Spartina densiflora* salt marshes (Spivak *et al.* 2001). These crabs are mainly deposit feeders (they feed on sediment with polychaetes, diatoms, ostracods, and nematodes) in the mudflats and herbivores in the *Spartina*-dominated areas (Iribarne *et al.* 1997; Bortolus and Iribarne 1999). Given that *Spartina spp.* are

probably the most important source of primary production in most marshes, the high density of crabs and the important role that deposit feeding has on sediment composition, this burrowing crab is a critical species in marsh production and marsh integrity (Bortolus and Iribarne 1999).

In spite of the aforementioned studies, the morphology of the claw closer muscle of *N. granulata* is still unknown. Therefore, morphological studies of *N. granulata* claw closer muscles will contribute to the understanding of fundamental facts in the field such as its behavior during mating, feeding, and also inter- and intraspecific competition in brackish salt marsh ecosystem of eastern South America.

In this work, we study the histological, histochemical, and morphometrical characteristics of the claw closer muscle of *N. granulata*.

Materials and Methods

Animals

Adult male crabs were collected at mudflats of Mar Chiquita Lagoon (37°32′S, 57°26′W), Province of Buenos Aires, Argentina. We selected crabs with claws of similar size (homochelae). They were transported to the laboratory in containers filled with water from the collection site. Then, they were acclimated for 10 days in aquaria with continuous aeration and controlled conditions of temperature 22 ± 2 °C, salinity 35 psu, and photoperiod 12 : 12 (L:D). The crabs, starved 48 h prior to the experiments, were fed with commercial food (Cichlind T.E.N.; Wardley, Secaucus, NJ, USA) three times a week. The molt stage was determined through observation of setae from the maxilla (Moriyasu and Mallet 1986); intermoulting individuals were selected. Before processing, the specimens were cold anesthesized and their claws removed.

Histological characterization

Aqueous Bouin or Davidson solution was injected into the closer muscle. The cuticle was removed and the muscle mass immersed in the same fluid for 24 h. The tissue was dehydrated in graded series of ethanol. Some samples followed the routine protocol (xylene-paraffin), whereas others were placed in butyl alcohol, butyl paraffin (50 : 50 v/v) and finally embedded in paraffin. Sections (5-μm thick) were cut and stained with hematoxylin-eosin (H-E), Mallory, Masson and Gomori trichromes, Gomori silver stain for collagen and reticular fibers, and Scarba Red for nuclei (Humason 1962; Martoja and Martoja-Pearson 1970).

Histochemical characterization

The animals were transferred to the cold room (4 °C). Claws were removed, their cuticles were extracted, and the closer muscles were fixed in liquid nitrogen (−170 °C). Cryosections (10–15 μm in thickness) of the central region of the muscle were subjected to histochemical techniques:

m-ATPase. To identify different types of fibers. Our procedures were modified from Guth and Samaha (1970) method. These modifications comprised (1) incubation at 4 °C; (2) incubation at room temperature (Mykles 1988); and (3) acid preincubation (acetate buffer, pH 4.35) and subsequent incubation, both at room temperature (Neil *et al.* 1993). The samples were incubated for 30 min. A control procedure with sodium glycerophosphate in place of ATP was carried out.

Succinate Dehydrogenase (SDH). To detect mitochondria. A modified Deffendi and Pearson (1955) method was developed: sections were incubated on moist filter paper, in a Petri dish, for 1–2 h at 40 °C (Neil *et al.* 1993). Controls included incubations without the sodium succinate substrate (Tse *et al.* 1983).

Periodic Acid Schiff (PAS) (Mc Manus 1948). To evidence glycoconjugates with oxidizable vicinal diols and/or glycogen. The samples were processed with periodic acid for 15 or 30 min. They were washed in running tap water, and then, they were stained with Schiff reagent for 2–5 min. As a control, the procedure was carried out after treatment of the sections with alpha-amylase for 45 min.

Sarcomere length

The closer muscle was fixed in a resting state by holding the dactyl partly open while Bouin's watery solution was injected through the exoskeleton (Govind and Blundon 1985; Govind *et al.* 1986). The cuticle was then removed and the muscle immersed in the same fluid for 24 h. The tissue was stored in ethanol 70%. The closer muscle was divided into three zones: dorsal, medial, and ventral. Six fiber bundles were isolated from the apodeme at random by each of these zones and split longitudinally into single fibers under a light microscope with a phase contrast (Chapple 1983; Taylor 2000). Three series of six consecutive sarcomeres and three series of three A-bands were measured in each muscle fiber using an ocular micrometer (Carl Zeiss Jena NU2). A-band and sarcomere lengths were measured in the same sarcomere. Therefore, both mean sarcomere and A-band lengths for a given fiber were the average of 18 sarcomeres and 9 A-bands, respectively. The A-band measurements were taken to verify that the sarcomeres were not stretched or contracted (Claxton *et al.* 1994; Fernández Giménez *et al.* 2007). The A-bands correspond to thick filaments and, thus, provide a very reliable measure of the size of the contractile unit in relation to the sarcomeres, which could present variable lengths depending on the state of the muscle contraction (Taylor 2000).

Right and left claws of three individuals were analyzed. Comparisons were made between the two whole closer muscles and between zones of the paired muscles of each animal.

The sarcomere length of both claws was contrasted by the Kolmogorow–Smirnov's (K–S) nonparametric test for two series of data (Lang *et al.* 1977b; Sokal and Rohlf 1979; Govind and Blundon 1985). A-bands and sarcomere lengths of every claw were compared through lineal regression analysis (Taylor 2000).

Results

Histological characterization

The tegumental tissue surrounds both the claw opener and closer muscles. The integument consists of a cuticle composed of proteins and chitin secreted by the underlying epithelium. The epithelium is columnar and single layered. Cells present basal nuclei and apical vacuoles. A loose connective tissue with abundant melanin underlies the epithelium (Fig. 1A).

Both the apodeme of the closer muscle and the integument possess similar histological characteristics. Muscle fibers attach to the apodeme at an angle (bipinnate attachment).

The connective tissue surrounds the individual fibers and the groups of myofibers, forming the endomysium and the perimysium, respectively. Abundant vessels and hemolymph sinuses generally associated with nerves appear in the latter.

The connective tissue consists of cells and an extracellular matrix (Fig. 1B). Different cell types are observed: typical connective cells and cells belonging to the immune system. The connective cells are fibroblasts that synthesize the extracellular matrix and can be inactive (fibrocytes). Both cell types are present in the perimysium and endomysium. The immune cells include hyaline hemocytes and granulocytes. Hyaline hemocytes are round-shaped and small, with scarce cytoplasm and a round central nucleus containing loose chromatin. Granulocytes have a granular acidophillic cytoplasm; they are bigger than hyaline hemocytes and present a lower nucleus : cytoplasm ratio and a more basophillic nucleus. Some granulocytes have a central nucleus, whereas others present an eccentric nucleus. The hemocytes are constituents of the perimysium (Fig. 1B).

The extracellular matrix includes fibers and an amorphous substance. Collagen fibers appear in the connective tissue of the integument and apodeme, as well as surrounding the fascicles of muscle fibers. Each myofiber is mainly surrounded by reticular fibers. The amorphous substance is evident amongst muscle cells.

Muscle fibers present a conspicuous striation. In the sarcomeres, I- and A-bands and the Z disk are clearly differentiated (Fig. 1C). Each muscle fiber contains numerous oval-elongated nuclei located peripherally along the cell. They are composed of loose chromatin. The cytoplasm is acidophillic (Fig. 1C, D).

Histochemical characterization

The m-ATPase of the claw closer muscle shows moderately contracting fibers in treatments with no preincubation. The stain intensity is uniform either for incubations at 4 °C

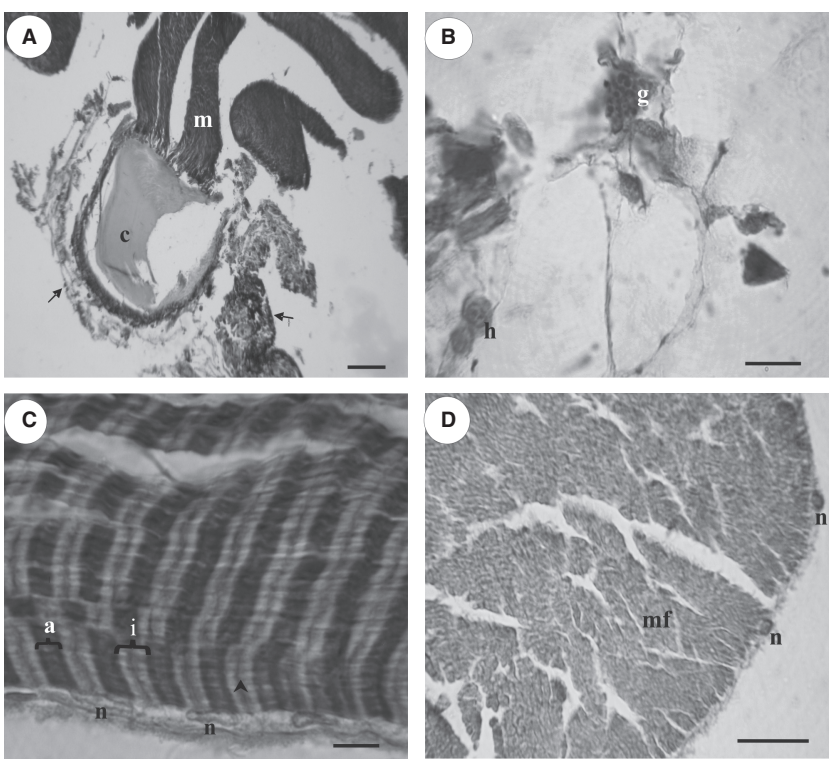

Fig. 1—Histological staining of the claw closer muscle of *Neohelice granulata*. —**A**. Insertion of closer muscle fibers on the cuticle (Masson Trichome). —**B**. Hemocytes in the perimysium (Masson Trichome). **C**.— Longitudinal section of the closer muscle showing the transverse striation (Gomori Trichome). **D**.— Transverse section of the closer muscle showing myofibrils and nuclei (H-E). m, muscle fiber; c, cuticle; arrow, subepithelial connective tissue; h, hyaline hemocyte; g, granular hemocyte; arrowhead, z disk; a, A-band; i, I-band; n, nucleus of muscle fiber; mf, myofibrils. Scale bars: —A 75 μm, B —12 μm, C —8 μm, D —20 μm.

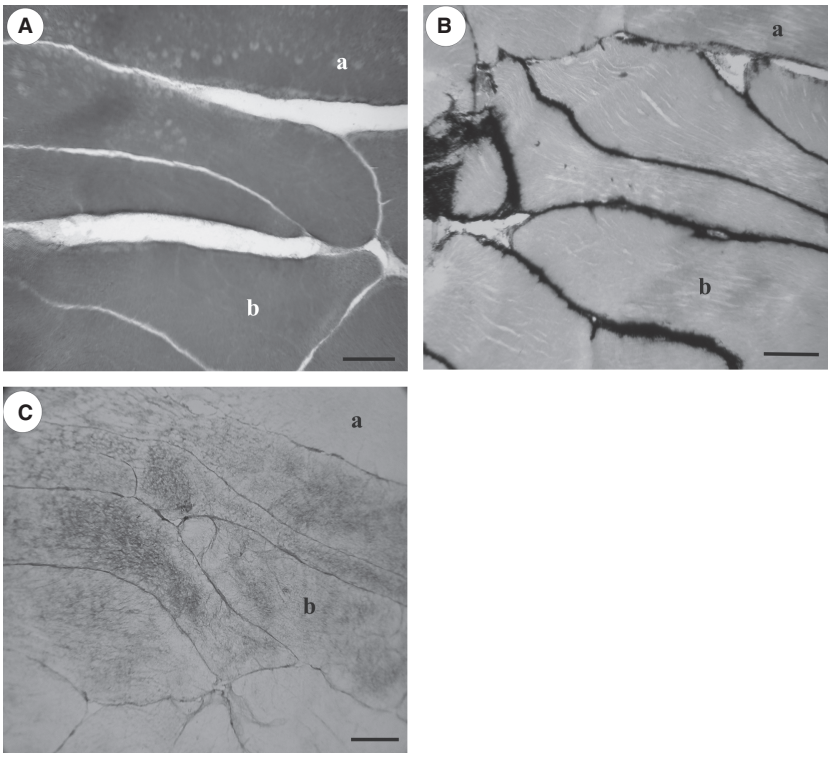

Fig. 2—Histochemical staining of the claw closer muscle of *Neohelice granulata*. —**A**. m-ATPase activity at 4°C. All fibers show the same stain intensity. —**B**. Succinate dehydrogenase activity. Fibers of moderate (a) and strong reaction (b) are present. —**C**. Periodic acid Schiff (PAS) staining. A fibers are negative, and B fibers react weakly to PAS. a: A type, b: B type. Scale bars: —A 170 μm, —B 175 μm, —C 150 μm.

(Fig. 2A) or at room temperature. Preincubation in acid solution (pH = 4.35) clearly evidences two types of muscle fibers: weak reaction fibers (acid-labile) and strong activity fibers (acid-stable). The acid-labile (A type) fibers have larger diameters and prevail in cross-sections whereas the acid-stable (B type) fibers are scarce and smaller.

Succinate dehydrogenase activity is subsarcolemmal, and two types of fibers are found: A and B, of moderate and strong reaction, respectively (Fig. 2B).

The glycogen content of muscle fibers, revealed by the PAS method, is null in A fibers. B fibers are feeble to PAS (Fig. 2C). Each muscular fiber is surrounded by an external PAS-positive layer (basal laminae). The amorphous substance among muscle cells and granules from granulocytes are also PAS-positive.

A summary of the histochemical profile of the claw closer muscle of *N. granulata* is given in Table 1.

Table 1 Histochemical profile of the claw closer muscle of *Neohelice granulata*

	Fiber type (by coloration)	
	A	B
m-ATPase (no preincubation/ incubation at 4 °C)	Moderate	Moderate
m-ATPase (no preincubation/ incubation at room temperature)	Moderate	Moderate
m-ATPase (acid preincubation/ incubation at room temperature)	Weak	Strong
Oxidative enzymes (SDH) (40 °C)	Moderate	Strong
Polysaccharides (PAS) (room temperature)	Negative	Weak

PAS, periodic acid Schiff; SDH, succinate dehydrogenase.

Sarcomere length

The claw closer muscle exhibits a heterogeneous composition of fiber types: short, intermediate, and long sarcomere fibers (SS, IS, and LS, respectively). LS fibers are predominant in the paired claw closer muscles of all individuals. Both claw muscle fiber populations show significant differences (K–S two sample test, at the 0.05 level). This is true for comparisons between the whole paired muscles (Fig. 3) as well as between each zone of the paired muscles of each animal (data not shown).

The percentage composition of the fiber types according to the sarcomere length for each individual is shown in Table 2. The LS fibers ratio decreases from the dorsal area toward the ventral zone of the muscle, whereas the IS and SS fibers ratio increases, following both claws a similar distribution.

The sarcomere length range of the whole claw from specimen number 1 varies between 2.4 and 12 μm. The sarcomere length values follow a bimodal distribution with the first mode at 3.2–4 μm and the second one at 10 μm (Fig. 4A.).

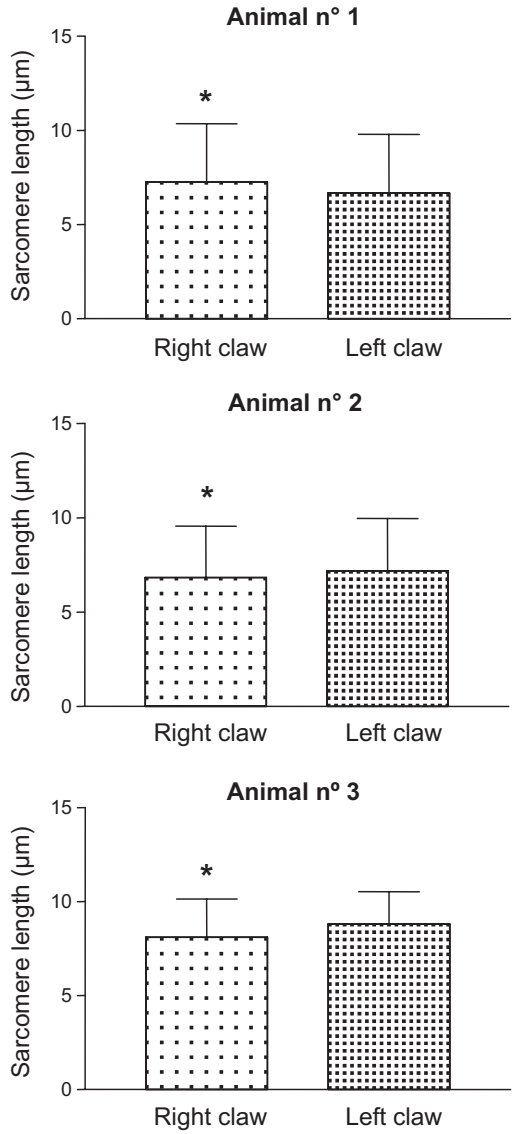

Fig. 3—Sarcomere length (mean ± SE) of the whole paired claw closer muscles. *Significantly different from the values of the left claw ($P < 0.05$).

The A-band length data shows the same distribution pattern as the sarcomere length, with values from 1 to 7.2 µm in specimen number 1. Mode values are equal to 1.6 µm and 4–6 µm (Fig. 4B). In all individuals, the sarcomere length and the A-band length show a linear regression with R^2 values between 0.7427 and 0.9574.

The mode values of the sarcomere length vary among the different zones of the muscle (Fig. 5).

Discussion

The histology of the claw closer muscle of *N. granulata* agrees with observations made on other crabs and decapod crustaceans (Atwood 1973; Johnson 1980; Chapple 1982; Hose *et al.* 1990; Martin and Hose 1992; Mellon 1992; Kondo *et al.* 1998; To *et al.* 2004).

Morphological, physiological, and histochemical criteria divide crustacean muscle fibers into two main categories: fast and slow (Neil *et al.* 1993). A continuum of intermediate fibers exists between the extreme groups (Atwood 1973; Mellon 1992).

The histochemical techniques characterize the muscle fibers in the claw closer muscle of *N. granulata*. As muscle fibers are moderately stained with the m-ATPase method without preincubation, they must be slow fibers. Two types of slow fibers have been differentiated according to the remaining tests. The A type shows acid-labile m-ATPase activity, moderate SDH activity, and no glycogen. The B type exhibits an acid-stable m-ATPase activity, strong reaction to SDH and low glycogen contents. In *N. granulata*, the results corresponding to SDH activity and m-ATPase activity with acid preincubation are similar to those reported for abdominal superficial flexor muscles of the lobster *Nephrops norvegicus*. In the lobster, the most oxidative type, with an m-ATPase activity resistant to acids, belongs to 'slow tonic' fibers (S_2), and the remaining type, to 'slow twitch' (S_1) (Neil *et al.* 1993). As in the present study, several authors have demonstrated subsarcolemmal oxidative enzymes activity (Silverman and Charlton 1980; Tse *et al.* 1983; Mykles 1988; Neil *et al.* 1993). This is consistent with the ultrastructural localization of mitochondria in the crustacean muscle fibers (Silverman and Charlton 1980). Mykles (1988) described two types of slow fibers in the claw closer muscle of the land crab *Gecarcinus lateralis*. The S_1 (twitch) type shows weak reaction to NADH-diaphorase and an m-ATPase activity slightly higher than S_2 (tonic) type at room temperature. Unlike those described in this work, both types could be differentiated with the m-ATPase method at room temperature, without preincubation. This differentiation is not conspicuous, for some S_2 fibers have m-ATPase activities similar to S_1 fibers. S_2 fibers present smaller diameters and are scarce in cross-sections (Mykles 1988; Neil *et al.* 1993). The same pattern of 'B' fibers is observed in *N. granulata*.

The most oxidative fibers of a swimming muscle from the blue crab show high glycogen contents (Tse *et al.* 1983). We corroborate the observation in *N. granulata* claw closer muscle.

The claw closer muscle of *N. granulata* is a mixed muscle made up of SS, IS, and LS fibers, being the LS the prevailing fibers in both claws. Fiber types follow a similar distribution pattern in the paired muscles: LS fibers ratio decreases from the dorsal area toward the ventral zone, whereas the IS and SS fibers ratio increases. In *Cyrtograpsus angulatus* (Varunidae), a crab that coinhabits in Mar Chiquita Lagoon, a distribution pattern of fiber types is also found in the closer muscle. The species presents IS and SS fibers only at the medial region of the muscle (Fernández Giménez *et al.* 2007).

Table 2 Percent distribution of muscle fiber types in the paired claw closer muscles of *Neohelice granulata*

		Sarcomere length (μm)					
		Right claw			Left claw		
		Short	Intermediate	Long	Short	Intermediate	Long
Animal	Muscle zones	<4	4–6	>6	<4	4–6	>6
1	Dorsal	5.6%	11.1%	83.3%	7.4%	25.9%	66.7%
	Medial	11.1	38.9	50	33.3	16.7	50
	Ventral	11.1	50	38.9	38.9	22.2	38.9
2	Dorsal	5.6	38.9	55.5	5.6	22.2	72.2
	Medial	16.7	44.4	38.9	5.6	38.9	55.5
	Ventral		Not measured		16.7	44.4	38.9
3	Dorsal	0	22.2	77.8	0	0	100
	Medial	5.6	16.7	77.7	0	5.6	94.4
	Ventral	0	27.8	72.2	5.6	18.5	75.9

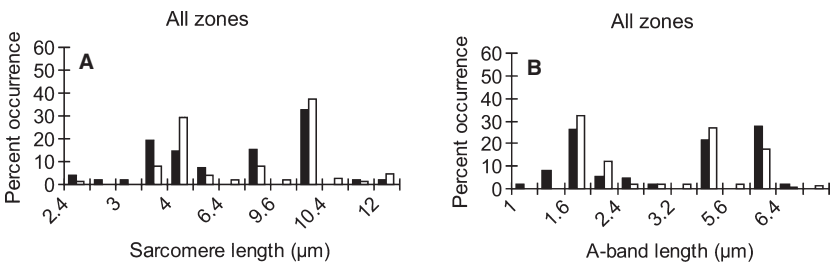

Fig. 4—Frequency histograms of sarcomere length and A-band length of the claw closer muscle of animal number 1. —**A**. Sarcomere length. —**B**. A-band length. Data for the entire muscle. Black bars: left claw; white bars: right claw.

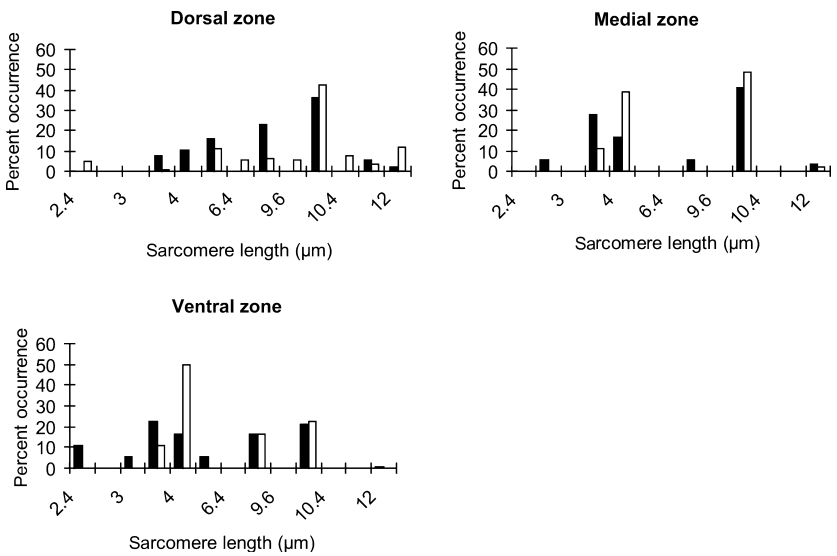

Fig. 5—Frequency histograms of sarcomere length of the claw closer muscle of animal number 1. Data for different zones of the muscle. Black bars: left claw; white bars: right claw.

Several authors have demonstrated a negative correlation between the sarcomere length and the m-ATPase activity of the muscle fibers: SS fibers are intensely stained with the m-ATPase method, while LS fibers react weakly to this technique (Silverman and Charlton 1980; Stephens *et al.* 1984). LS fibers of *N. granulata* prevail, and this fact agrees with the

moderate stain intensity of fibers to the m-ATPase test. Both features are characteristic of the 'slow' fibers.

Some decapod crustaceans have one of the chelae more developed than the other (heterochelae). Claw asymmetry is often linked to a dimorphism of the closer muscle composition that has been associated with a functional asymmetry, in the lobster *Homarus americanus*, the shrimps *Alpheus spp*., the hermit crab *Pagurus pollicaris*, and the male fiddler crabs *Uca spp*., but not in the blue crab *Callinectes sapidus* (Lang *et al.* 1977a; O' Connor *et al.* 1982; Stephens *et al.* 1984; Govind and Blundon 1985; Mykles 1988). Strikingly, in the present study, we found significant differences in the closer muscle composition of *N. granulata* symmetrical claws: both claws present closer muscles with diverse types of fibers, being slow A fibers (low resistant to fatigue) predominant.

Further works will be needed to extend the morphological study of the claw closer muscle of *N. granulata* through the use of ultrathin sections.

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References

Atwood, H. L. 1973. An attempt to account for the diversity of crustacean muscles. – *American Zoologist* **13**: 357–378.

Bortolus, A. and Iribarne, O. 1999. Effects of the SW Atlantic burrowing crab *Chasmagnathus granulata* on a *Spartina* salt marsh. – *Marine Ecology Progress Series* **178**: 79–88.

Chapple, W. D. 1982. Muscle. In: Bliss, D. E., Atwood, H. L. and Sandeman, D. C. (Eds): *The Biology of Crustacea*, vol. 3, pp. 151–184. Academic Press, New York.

Chapple, W. D. 1983. Mechanical responses of a crustacean slow muscle. – *Journal of Experimental Biology* **107**: 367–383.

Claxton, W. T., Govind, C. K. and Elner, R. W. 1994. Chela function, morphometric maturity, and the mating embrace in male snow crab, *Chionoecetes opilio*. – *Canadian Journal of Fishery and Aquatic Sciences* **51**: 1110–1118.

Deffendi, V. and Pearson, B. 1955. Quantitative estimation of succinic dehydrogenase activity in a single microscopic tissue section. – *Journal of Histochemistry and Cytochemistry* **3**: 61–69.

El Haj, A. J. and Whiteley, N. M. 1997. Molecular regulation of muscle growth in crustacea. – *Journal of the Marine Biological Association of the United Kingdom* **77**: 95–106.

Fernández Giménez, A. V., Goldemberg, A. L. and Díaz, A. O. 2007. Studies of the claws of the crab *Cyrtograpsus angulatus* Dana (Crustacea, Brachyura, Grapsidae). – *Biociencias* **15**: 29–39. (On line). Brazil.

Govind, C. K. 1984. Development of asymmetry in the neuromuscular system of lobster claws. – *Biological Bulletin* **167**: 94–119.

Govind, C. K. and Blundon, J. A. 1985. Form and function of the asymmetric chelae in blue crabs with normal and reversed handedness. – *Biological Bulletin* **168**: 321–331.

Govind, C. K. and Lang, F. 1978. Development of the dimorphic claw closer muscles of the lobster *Homarus americanus*: III. Transformation to dimorphic muscles in juveniles. – *Biological Bulletin* **154**: 55–67.

Govind, C. K., Quigley, M. M. and Mearow, K. M. 1986. The closer muscle in the dimorphic claws of male fiddler crabs. – *Biological Bulletin* **170**: 481–493.

Günzel, D., Galler, S. and Rathmayer, W. 1993. Fibre heterogeneity in the closer and opener muscles of crayfish walking legs. – *Journal of Experimental Biology* **175**: 267–281.

Guth, L. and Samaha, F. J. 1970. Procedure for the histochemical demonstration of actomyosin ATPase. – *Experimental Neurology* **28**: 365–367.

Hose, J. E., Martin, G. G. and Sue Gerard, A. 1990. A decapod hemocyte classification scheme integrating morphology, cytochemistry, and function. – *Biological Bulletin* **178**: 33–45.

Humason, G. L. 1962. *Animal Tissue Techniques*, pp. 140–141. W. H. Freeman and Company, San Francisco.

Iribarne, O., Bortolus, A. and Botto, F. 1997. Between-habitat differences in burrow characteristics and trophic modes in the southwestern Atlantic burrowing crab *Chasmagnathus granulata*. – *Marine Ecology Progress Series* **155**: 137–145.

Johnson, P. T. 1980. *Histology of the Blue Crab,* Callinectes sapidus. *A Model for the Decapoda*. Praeger Publishers, New York.

Kondo, M., Itami, T., Takahashi, Y., Fujii, R. and Tomonaga, S. 1998. Ultrastructural and cytochemical characteristics of phagocytes in kuruma prawn. – *Fish Pathology* **33**: 421–427.

Lang, F., Costello, W. J. and Govind, C. K. 1977a. Development of the dimorphic claw closer muscles of the lobster *Homarus americanus*: I. Regional distribution of muscle fiber types in adults. – *Biological Bulletin* **152**: 75–83.

Lang, F., Govind, C. K. and She, J. 1977b. Development of the dimorphic claw closer muscles of the lobster *Homarus americanus*: II. Distribution of muscle fiber types in larval forms. – *Biological Bulletin* **152**: 382–391.

Lee, S. Y. 1995. Cheliped size and structure: The evolution of a multi-functional decapod organ. – *Journal of Experimental Marine Biology and Ecology* **193**: 161–176.

Luppi, T. A., Spivak, E. D. and Anger, K. 2001. Experimental studies on predation and cannibalism of the settlers of *Chasmagnathus granulata* and *Cyrtograpsus angulatus* (Brachyura: Grapsidae). – *Journal of Experimental Marine Biology and Ecology* **265**: 29–48.

Martin, G. G. and Hose, J. E. 1992. Vascular elements and blood (hemolymph). In: Harrison, F. W. and Humes, A. G. (Eds): *Microscopic Anatomy of Invertebrates, Decapod Crustacea*, vol. 10, pp. 117–146. Wiley-Liss, New York.

Martoja, R. and Martoja-Pearson, M. 1970. *Técnicas de Histología Animal*. Ed. Toray-Masson, S.A.

Mc Manus, J. F. A. 1948. Histological and histochemical uses of periodic acid. – *Stain Technology* **23**: 99–108.

Mellon, F. Jr 1992. Connective tissue and supporting structures. In: Harrison, F. W. and Humes, A. G. (Eds): *Microscopic Anatomy of Invertebrates, Decapod Crustacea*, vol. 10, pp. 77–116. Wiley-Liss, New York.

Moriyasu, M. and Mallet, P. 1986. Molt stages of the snow crab *Chionoecetes opilio* by observation of morphogenesis of setae on the maxilla. – *Journal of Crustacean Biology* **6**: 709–718.

Mykles, D. L. 1988. Histochemical and biochemical characterization of two slow fiber types in decapod crustacean muscles. – *Journal of Experimental Zoology* **245**: 232–243.

Neil, D. M., Fowler, W. S. and Tobasnick, G. 1993. Myofibrillar protein composition correlates with histochemistry in fibres of the abdominal flexor muscles of the Norway lobster *Nephrops norvegicus*. – *Journal of Experimental Biology* **183**: 185–201.

O' Connor, K., Stephens, P. J. and Leferovich, J. M. 1982. Regional distribution of muscle fiber types in the asymmetric claws of Californian snapping shrimp. – *Biological Bulletin* **163**: 329–336.

Silverman, H. and Charlton, M. P. 1980. A fast-oxidative crustacean muscle: Histochemical comparison with other crustacean muscle. – *Journal of Experimental Zoology* **211**: 267–273.

Sokal, R. R. and Rohlf, F. J. 1979. *Biometría. Principios y métodos estadísticos en la investigación biológica*. Primera edición en español, H. Blume Ediciones.

Spivak, E., Luppi, T. and Bas, C. 2001. Cangrejos y camarones: Las relaciones organismo-ambiente en las distintas fases del ciclo de vida. In: Iribarne, O. (Ed.): *Reserva de Biosfera de Mar Chiquita: Características físicas, biológicas y ecológicas*, pp. 129–151. Editorial Martín, Mar del Plata.

Stephens, P. J., Lofton, L. M. and Klainer, P. 1984. The dimorphic claws of the hermit crab, *Pagurus pollicaris:* Properties of the closer muscle. – *Biological Bulletin* **167**: 713–721.

Taylor, G. M. 2000. Maximum force production: Why are crabs so strong?. – *Proccedings of the Royal Society of London B* **267**: 1475–1480.

To, T. H., Brenner, T. L., Cavey, M. J. and Wilkens, J. L. 2004. Histological organization of the intestine in the crayfish *Procambarus clarkii*. – *Acta Zoologica* **85**: 119–130.

Tse, F. W., Govind, C. K. and Atwood, H. L. 1983. Diverse fiber composition of swimming muscles in the blue crab: *Callinectes sapidus*. – *Canadian Journal of Zoology* **61**: 52–59.