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ULTRASTRUCTURAL STUDY OF SKELETAL MUSCLE FIBBER TYPES FROM *Caquetaia kraussii* AND *Colossoma macropomum*.

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RESUMEN: El propósito de este trabajo fue el de determinar el tipo de fibra muscular presente en la musculatura esquelética de dos especies de peces tropicales (*Caquetaia kraussii* y *Colossoma macropomum*). Muestras de los músculos caudales y dorsales fueron procesadas por las técnicas convencionales para la Microscopía Electrónica de Transmisión (MET). En los músculos dorsales y caudales de *C. kraussii* y *C. macropomum* se encontraron solo fibras fásicas con características intermedias entre las fibras fásicas IIa (rosadas) y IIb (blancas). El grosor de la línea Z fue similar al de las fibras IIb (de aproximadamente 50 nm). El sistema sarcotubular en este tipo de fibra correspondió por su buen desarrollo al de ambos tipos de fibras. Sin embargo, no se apreciaron gotas lipídicas. La composición en los tipos de fibras en los músculos estudiados podría corresponder a desafíos fisiológicos y del medio ambiente tales como disponibilidad de nutrientes y presencia de depredadores dada la gran adaptabilidad y plasticidad del músculo esquelético.

Palabras claves: *Caquetaia kraussii*, *Colossoma macropomum,* ultraestructura muscular.

ABSTRACT: The aim of this work was to determine the muscle fibber type that comprises the skeletal musculature of two tropical species of fishes (*Caquetaia kraussii* and *Colossoma macropomum*). Samples of caudal and dorsal muscles were studied by Transmission Electron Microscopic Techniques (TEM). In both, *C. kraussii* and *C. macropomum,* caudal and dorsal muscles, were found only one fibber type with intermediate characteristics between IIa (pink) and IIb (white) twitch fibbers. The Z line thickness was similar to that of IIb fibbers (approximately 50 nm). The sarcotubular system presented a well development and corresponded to both types of twitch fibbers. Lipid droplets of IIa type of fibber were not found. The composition in fibber types of studied muscles could respond to environmental and physiological challenges as well as nutrient availability and predators due to high adaptability and plasticity of skeletal muscle.

Key words: *Caquetaia kraussii*, *Colossoma macropomum*, muscle ultrastructure.

INTRODUCTION

The locomotion musculature in a great amount of teleost fishes comprises 60% of the total mass, giving also the power for a quick swimming (Sanger & Stoiber 2001). The skeletal muscle of vertebrate consists of tonic fibbers which are specialized for slow steady contraction and twitch fibbers, specialized for rapid movements (Hess 1970). The twitch skeletal muscle may show three different types of fibbers (Finol & Ogura 1972, 1977; Prochaska *et al*. 1982; Kiessling *et al*. 2006): a) slow twitch fibber (type I) contract slowly and fatigue slowly. They are used both for maintaining posture and for moderately fast, repetitive movements, because they contain a large number of mitochondria and depend on oxidative phosphorylation for obtaining ATP. Also they use this molecule at a relatively slow rate. This type of fibber is usually called red because it contains a high concentration of myoglobin which proportions to muscle its red color. b) The second twitch fibber type is the fast oxidative (Type IIa) fibber specialized for rapid, repetitive

movements as in sustained, strenuous locomotion. The fibbers have many mitochondria and can produce ATP quickly by oxidative phosphorylation and thus fatigue slowly. c) The last are the fast quickly glycolytic (type IIb) fibbers that contract rapidly and fatigue quickly. The fibbers are generally recruited when a very rapid contraction is required. They contain few mitochondria and thus depend on anaerobic glycolysis to generate ATP. These categories are somewhat arbitrary, because some muscle fibbers combine properties of different types. Also, the absolute values for many of the parameters may vary along species. Within a given muscle, however, the fibber types can be distinguished by their histological properties. Another useful method is based on the abundance of oxidative enzymes such as succinic dehydrogenase (Stein & Padykula 1962). Until now, there is limited information about muscle ultrastructure of continental fishes. The aim of this work was to determine muscle fibber type that holds the skeletal musculature of two species: *Caquetaia kraussii* (Pisces, Ciclidae) which is a great food source for its excellent flavour, not very bony axial skeleton, low fat and high protein contents (Segnini & Chung 2001) and *Colossoma macropomum* (Pisces, Characeae), a species widely found in South America from the Orinoco basin to the Amazon basin river. In Venezuela, it is abundant in the Guanare, Portuguesa, Meta, Apure, Caroni, and Orinoco rivers. Also, they have a high aquaculture potential because they can be cultured and reproduced in captivity (Gonzalez & Heredia 1998).

MATERIALS AND METHODS

The specimens of *Caquetaia kraussii* and *Colossoma macropomum* came from fishes cultivated at the experimental Station Guanapito, an experimental center of the National Fund for Agricultural Investigations (FONAIP), Guárico State, Venezuela. 8 fishes from each species were selected. After that, fishes were decapitated and samples of 1x2mm of caudal and dorsal muscles were fixed with 2.5% glutaraldeyde in Millonig buffer (pH = 7.8 and 329 mOsmol), postfixed with 1% OsO_4, dehydrated in increasing ethanol concentrations and embedded in resin LX-112 (Ladd Research, Inc, Burlington, USA). Ultrathin sections stained with uranyl acetate and lead citrate were observed in a Hitachi H-7100 TEM at an accelerating voltage of 75 kV.

RESULTS

The transmission electron microscopy is an analytical method used to characterize and identify muscle fibber types where the properties like Z line thickness, volume density and mitochondrial morphology, volume density and distribution of sarcotubular system (sarcoplasmic reticulum and T tubules) can be studied. In relation to the type of fibbers, both species showed an intermediate type with characteristics of white (IIb) and pink (IIa) fibbers. In *C. kraussii,* myofibrils presented sarcomeres with well organized bands (A, H and I) and lines (M and Z). Z lines were thin (50 + 22.04 nm, N = 72) and straight with triads situated at its level (Fig 1). Cross-sections at the level of A – I bands exhibited six thin filaments per one thick filament (Fig. 2 down). Mitochondria were seen around the nucleus at the subsarcolemmal space (Fig 2 upper) with tubular cisternae and electron dense matrix. Lipid droplets were not found.

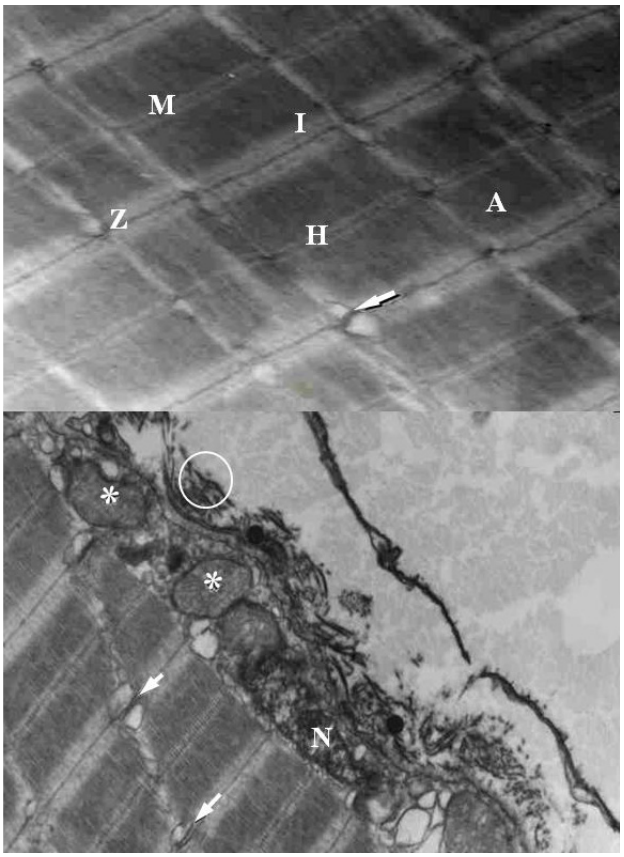


Fig. 1. Electron micrograph of the longitudinal section of *Caquetaia kaussii* skeletal muscle fibber. Upper: showing electron dense A-band with the central H-band bisected by M-line, electron transparent I-band bisected by Z-line and the triad located at the Z-line level (arrow). 33,000X. Down: notice subsarcolemmal nucleus (N), mitochondria (*) and collagen. 30,000X.

C. macropomum also exhibited an intermediate type of fibber with similar characteristics to that presented by *C. kraussii* skeletal musculature in relation to the development of contractile and sarcotubular systems, showing Z lines straight with approximately 50 nm of thickness, mitochondrial morphology and disposition (Figs. 3-4). Similarly, *C. macropomum* intermediate skeletal muscle fibber did not present lipid droplets.

DISCUSSION

In the caudal muscle of the tropical fish "coporo" (*Procilodus mariae*) was described a type of unusual skeletal muscle fibber presenting intermediate characteristics between twitch and tonic fibbers (Heredia & Finol 1999). In the present work, using caudal and dorsal muscles of *C. kraussii* and *C. macropomum* we observed the presence of a type of fibber with an ultrastructure intermediate between IIa (pink) and IIb (white) fast twitch fibbers. In *P. mariae* and in *C. kraussii* and *C. macropomum* the intermediate type of fibber presented a Z line thickness similar to that of IIb fibbers as in white fibbers described in different vertebrades (Finol & Ogura 1972, 1977; Finol 1978, 1980; Finol & Colina 1979; Akster 1981; Prochaska *et al*. 1982).

In fishes reported values have been contradictory in relation to the Z line thickness and fibber type described depending on species. In red and pink fibbers from carp (*Cyprinus carpio*), Z line had a thickness of approximately 100 nm (Akster 1985), value different from that reported for perch (*Perca* fluviatilis), which were between 70 and 75 nm (Akster 1981). These last values are nearer to the value reported in other vertebrates. This is the reason why some authors avoid to measure Z line thickness and consider just that it is thick or fine (Kilarski 1967; Korneliussen & Nicolaysen 1973). The relation of six thin filaments per thick one seen in myofibril cross-sections at the A-I level has been described in IIa and IIb fibbers from other vertebrates (Huxley 1957; Prochaska *et al*. 1982). Similarly, the sarcotubular system presented a well developed sarcoplasmic reticulum forming with T tubules triads at the level of Z line as it was also described for the intermediate type of fibber found in *P. mariae* caudal muscle (Heredia & Finol 1999).

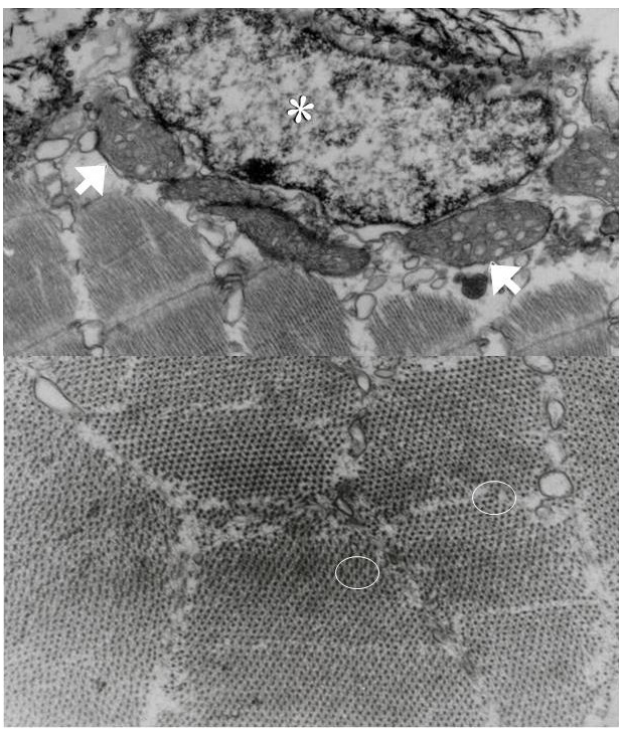


Fig. 2. Electron micrograph of section from *Caquetaia kraussii* skeletal muscle fibber. Upper: notice mitochondria (arrows) next to the nucleus (*). 30,000X. Down: Transversely sectioned skeletal muscle fibber from *Caquetaia kraussii*. Note areas of six thin filaments per one thick filament (circles). 60,000X.

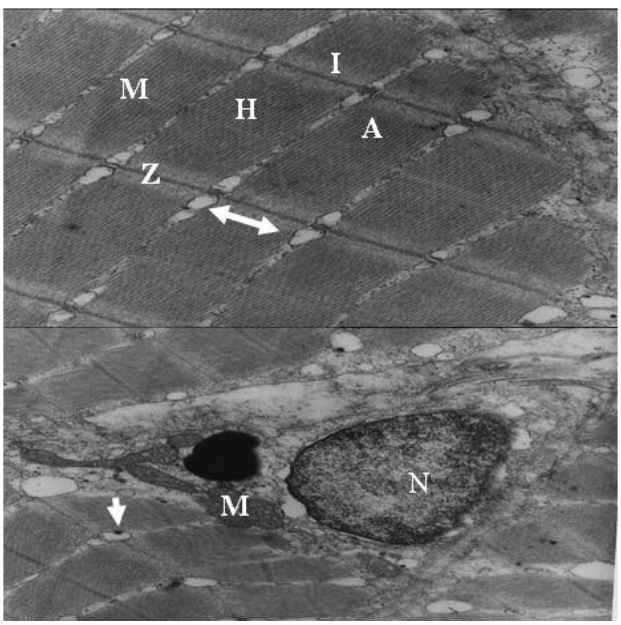


Fig. 3. Electron micrograph of longitudinal section of *Colossoma macropomum* skeletal muscle finer. Upper: showing electron dense A-band with the central H-band bisected by M-line and electron transparent I-band bisected by Z-line and triads located at the Z-line level. 45,000X. Down: notice a subsarcolemmal nucleus (N) next to mitochondria (M). Note calcium salts inside a terminal cisternae (arrow). 36,000X.

Other structures used for the characterization of skeletal muscle fibber types are lipid droplets and mitochondria, which are abundant in fish type I and type IIa fibbers (Kilarski 1967; Korneliussen & Nicolaysen 1973; Akster 1981, 1985; Domínguez *et al*. 1988; Heredia & Finol 1999). Until recently, lipid droplets were considered cellular inclusions or storage deposits, participating passively in energy homeostasis (Beller *et al*. 2010). Nowadays, lipid droplets have been considered as dynamic intracellular storage organelles (Digel *et al*. 2010) and even as multi-functional organelles that affect different aspects of the Biology of the Cell and its metabolism (Yand *et al*. 2012). Lipid droplets are composed of a hydrophobic core containing neutral lipids and cholesteryl esters surrounded by a phospholipid monolayer which also includes proteins of the PAT protein family perilipin, adipophilin, TIP47, S3-12 and OXPAT (Diegel *et al*. 2010; Buers 2011). In the skeletal muscle, intermediate type of fibber was found in the present work, lipid droplets were not observed. This could mean that the fibber used glycogen particles as fuel for obtaining ATP from glycolysis and oxidative phosphorylation process and supplying for muscle contraction.

It has been stated that an important difference between striated fish muscle and that found in higher vertebrates is a higher glycolytic and anaerobic type of fibber (IIb or white) that constitute about 90 – 95% of all muscles in most fish species (Kiessling *et al*. 2006) and intermediate (IIa or pink) fibbers that represent less than 10% of the myotomal musculature. In the case of salmonids, they seem not to have pink fibbers. In tropical fishes *P. mariae* (Heredia & Finol 1999) and *C. kaussii* and *C. macropomum* the type of fibber presented in dorsal and caudal muscles was a variety of intermediate twitch fibber according to the Z line thickness (fine Z line as in IIb fibbers), as well as a developed sarcotubular system and abundant mitochondria particularly in subsarcolemmal spaces (as in pink or IIa fibbers). These variations in type of fibbers could be related to the fact that the main object for fish muscle is movement unlike other vertebrates as mammals where muscles also are important in order to support skeleton (Kiessling *et al*. 2006). It is interesting that in slow - twitch oxidative (red or I) and fast - twitch glycolytic (white or IIb) fibbers from striped bass (*Morone saxatilis*), acclimated from 25 to 5 degrees C results in an increase in total mitochondrial volume per 100g body wt (Egginton & Sidell 1989). Nevertheless, the absence of IIa fibbers in fishes from northern regions results contradictory because that type of fibber is very important for movement because the presence of mitochondria, the arrangement of 6 thin filaments per one thick filament and the well development of the sarcotubular system would suggest that it is a more complete type of fibber having the possibility to be used for fast (short) and sustained movements. The mentioned arrangement of 6 thin per one thick filaments in a regular and hexagonal disposition could result more efficient in terms of development and amplitude of contraction as it could come out from theory of sliding of thin (actin) filaments among thick (myosin) ones (Hanson & Huxley 1953; Huxley & Hanson 1954; Huxley 1969).

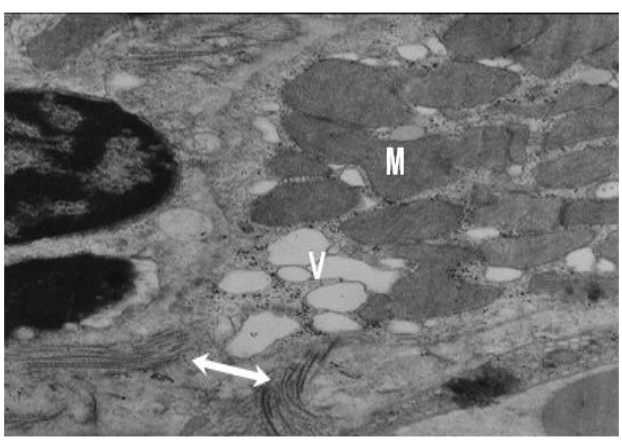


Fig. 4. Electron micrograph of section of *Colossoma* macropomum skeletal muscle fibber. Note abundant mitochondrial (M) and vacuoles (V) at the subsarcolemmal space and collagen (double head arrow). 24,000X.

The presence of an intermediate type of fibber between IIa and IIb fibbers as in *P. mariae*, *C. kraussii* and *C. macropomum* could be related to active migrations performed by these species in order to develop diverse activities as it has been pointed out by distinct investigations (Mago-Leccia 1978; Goulding 1980; Novoa & Ramos 1982). In conclusion, the use of transmission electron microscopy would represent an important tool in the study of skeletal muscle types of fibbers and its importance for the previously mentioned activities.

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