

# Myoarchitecture and vasculature of the heart ventricle in some freshwater teleosts

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## Abstract

The morphological characteristics of the ventricular myocardium and of coronary vascularization were studied in three freshwater teleost species, *Piaractus mesopotamicus*, *Colossoma macropomum* and *Clarias gariepinus* (African catfish), by correlating their ventricular shapes and swimming habits. In *Piaractus mesopotamicus* and *Colossoma macropomum*, species with highly active swimming habits, the cardiac ventricle showed a pyramidal shape and a richly vascularized myocardium consisting of an outer compact layer and inner spongy layer. In *Clarias gariepinus*, a less active species, we observed a saccular ventricle with a mixed myocardium and coronary arteries, in contrast to the ventricular structure of other species described in the literature.

**Key words** coronary arteries; morphology; myocardium.

## Introduction

The heart of the teleost fish is tubular and consists of four consecutive chambers: the venous sinus, atrium, ventricle and bulbus arteriosus (Godinho, 1970). The morphology of the cardiovascular apparatus in the fish has been relatively well studied, and the cardiac ventricle has been found to present three distinct shapes: saccular, tubular and pyramidal. These different ventricular forms are associated with two structurally different types of myocardium. The mixed ventricular myocardium is characterized by a cortical layer of variable thickness, compact layer and an internal spongy layer. In contrast, the trabecular (spongy) ventricular myocardium consists exclusively of trabecular muscle (Santer et al. 1983; Santer, 1985).

Mixed ventricular myocardium is found in the pyramidal ventricles of species with highly active swimming habits (Tota, 1978; Santer & Greer-Walker, 1980), while the trabecular ventricular myocardium is observed in

the saccular or tubular ventricles of inactive teleosts (Santer et al. 1983; Greer Walker et al. 1985; Santer, 1985).

Previous studies by the authors have indicated in teleost fish the existence of a relationship between locomotor activity, myoarchitecture and type of blood supply to the cardiac ventricle. Inactive species present saccular or tubular cardiac ventricles and a trabecular myocardium with lacunar venous circuits. Moreover, active species possess a large pyramidal cardiac ventricle with a mixed-type myoarchitecture, in addition to a well-developed coronary artery supply supporting the high metabolic demand of the organism (Santer & Greer Walker, 1980; Santer et al. 1983; Tota et al. 1983).

Elasmobranchs and about one-third of the teleosts studied possesses a relatively well-developed coronary circulation. However, the structure of the fish heart is not uniform, with variable distribution of the coronary arteries (Axelsson, 1995).

Based on these data, the objective of the present study was to describe the morphological characteristics of the ventricular myocardium and the coronary vascularization of the freshwater species *Piaractus mesopotamicus* (Characidae; Holmberg, 1887), *Colossoma macropomum* (Characidae; Cuvier, 1818) and *Clarias gariepinus* (Clariidae; Burchell, 1822), correlating their

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swimming habits with the heart ventricle type of each species.

## Materials and methods

The morphological characteristics of the ventricular myocardium and coronary vascularization were studied in 30 specimens per species of adult freshwater teleost fish, *Piaractus mesopotamicus* (1.430–1.550 kg), *Colossoma macropomum* (1.500–1.680 kg) and *Clarias gariepinus* (0.970–1.100 kg) obtained in the freshwater tanks of the Aquaculture Center of UNESP (CAUNESP). The animals were anaesthetized with MS 222 (Sandoz) and their coeloms were opened to expose the heart.

For anatomical analysis, the hearts were fixed in 10% neutral formalin for 24 h, dissected out, analysed and systematized with use of a camera lucida (Zeiss, Germany). To study the superficial ventricular vascularization, the coronary vessel system was perfused with red Dupont Neoprene latex (USA) slowly injected through a thin cannula inserted into the cranial portion of the bulbus arteriosus. The coronary arteries were perfused via their natural course through the gills and by the hypobranchial arterial system. Before injection, possible cardiac inflow channels were eliminated by ligating the venous sinus and atrium to prevent injection of the intertrabecular spaces (Tota et al. 1983). The hearts were then fixed in 10% neutral formalin for later analysis of the vasculature.

The distribution of coronary arteries in the compact layer was studied in hearts perfused with India ink through a cannula inserted into the cranial portion of the bulbus arteriosus. The hearts were removed, fixed in Bouin's solution for 24 h, dehydrated and diaphanized. The material was embedded in paraplast (Oxford-Labware, USA), sections 15–30 µm thick were obtained and stained with eosin.

For histological analysis of the ventricular myocardium, fragments of the ventricles were fixed in 10% buffered formalin for 24 h, processed and embedded in historesin (Leica, Germany). Sections 3 µm thick were stained with Hematoxylin-floxin B and 10% Toluidine blue.

For morphometric analyses of the compact layer thickness, 10 animals per species were used. Thirty points at the apex and on the ventricular border were measured in different histological sections from every heart using the KS-300 program (Zeiss). Data were analysed statistically by analysis of variance (ANOVA)

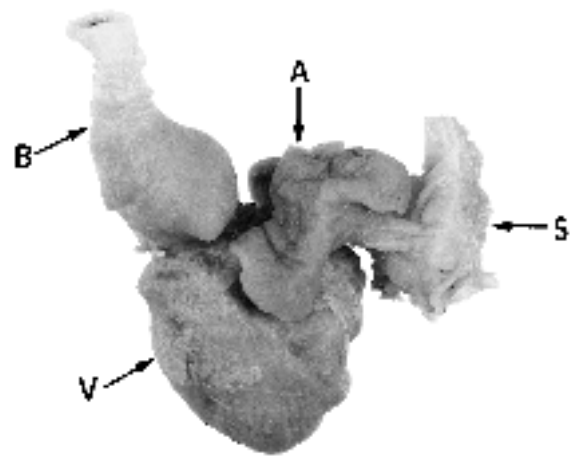


Fig. 1 Heart of *P. mesopotamicus* (ventral view) showing venous sinus (S), atrium (A), ventricle (V) and bulbus arteriosus (B) ( $\times 1.6$ ).

using the SAS® statistical program (Statistical Analyses System).

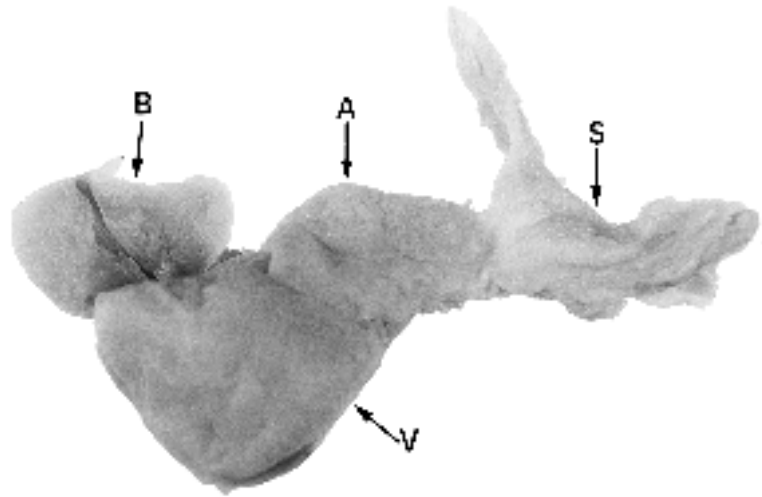
## Results

### Gross anatomy

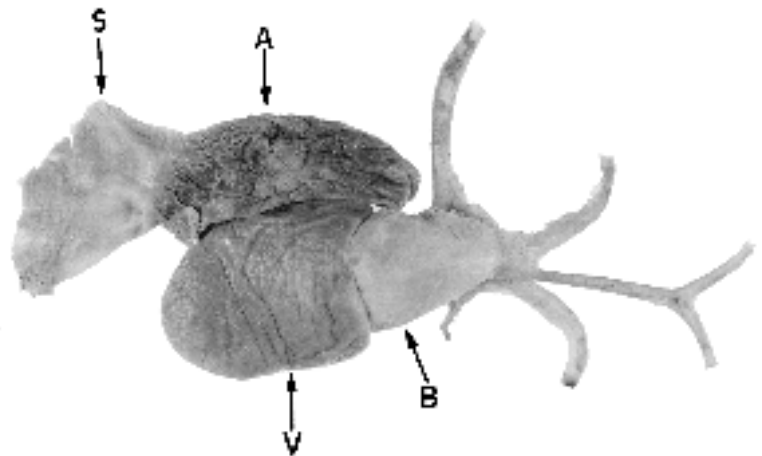
The hearts of the teleosts *Piaractus mesopotamicus*, *Colossoma macropomum* and *Clarias gariepinus* consisted of four consecutive chambers, the venous sinus, atrium, ventricle and bulbus arteriosus. To simplify the topography description of the hearts, we based our observations on the 'free-standing' hearts, orientated according to the longitudinal axis of the body. The ventricles presented different shapes, with a pyramidal shape observed in *P. mesopotamicus* (Fig. 1) and *C. macropomum* (Fig. 2), species with highly active swimming habits, and a saccular shape was observed in *C. gariepinus* (Fig. 3), a less active species. The pyramidal ventricle showed a triangular shape with well-defined angles. The saccular ventricle presented oval or spherical shapes and was flattened dorsoventrally (Fig. 3).

### Ventricular coronary vascularization

Differences on the superficial ventricular coronary vascularization were observed among the species studied in terms of number and distribution of the coronary arteries. *P. mesopotamicus* and *C. macropomum* possessed two main coronary arteries, one on the left and the other on the right, which descended on the lateral surface of the bulbus arteriosus. However, the left



**Fig. 2** Heart of *C. macropomum* (ventral view) showing venous sinus (S), atrium (A), ventricle (V) and bulbus arteriosus (B) ( $\times 1.6$ ).



**Fig. 3** Heart of *C. gariepinus* (ventral view) showing venous sinus (S), atrium (A), ventricle (V) and bulbus arteriosus (B) ( $\times 1.6$ ).

coronary artery descended laterally through the bulbus arteriosus and encircled the base of the atrium, giving branches to both chambers. Two main branches produced ramifications to both sides of the ventricle on the ventral and dorsal surfaces of the cardiac ventricle of *P. mesopotamicus*, at the atrioventricular junction level (Fig. 4).

In the atrioventricular region of *C. macropomum*, the right coronary artery comprised two main branches on the ventral surface and the other branch on the dorsal surface of the cardiac ventricle (Fig. 4). A single coronary artery was observed in *C. gariepinus*, which descended along the dorsal surface of the bulbus arteriosus, encircling it and branching to both surfaces of the ventricle and to the heart atrium. At the atrioventricular junction the coronary artery comprised two main branches, each on the ventral and dorsal surface of the ventricle. Free anastomoses of the smaller branches of the coronary arteries were observed on the

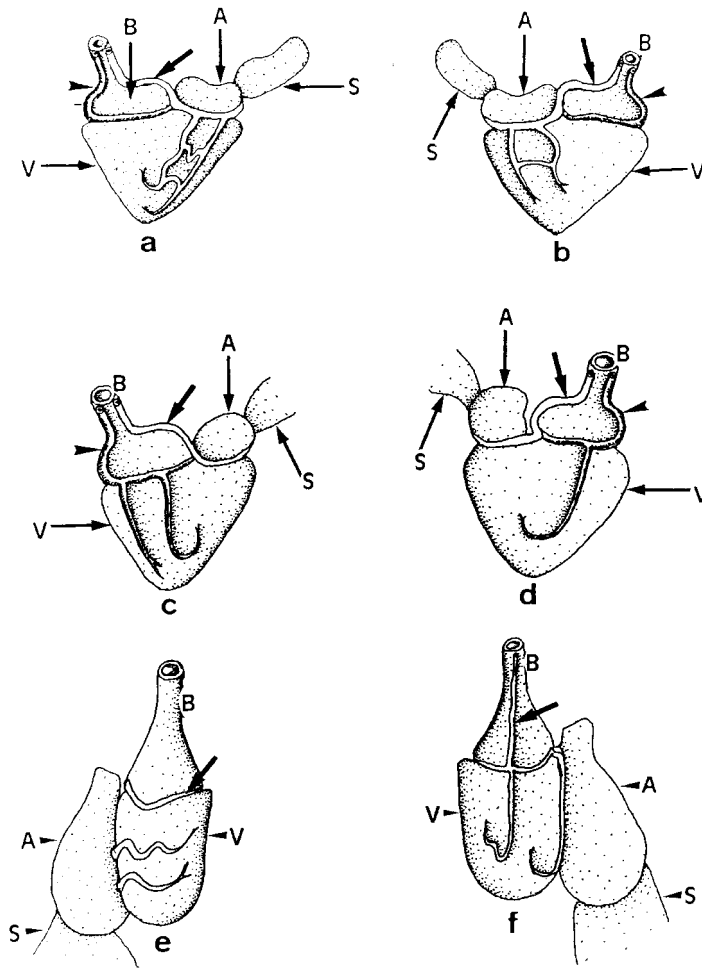
ventral and dorsal surfaces of the ventricles studied (Fig. 4).

With respect to the vascularization of the ventricular myocardium, we observed a uniform pattern in terms of distribution and ramification of coronary arteries in the compact layer. The three species have coronary arteries that create subepicardial arteries which branch extensively throughout the compact layer (Figs 5–7).

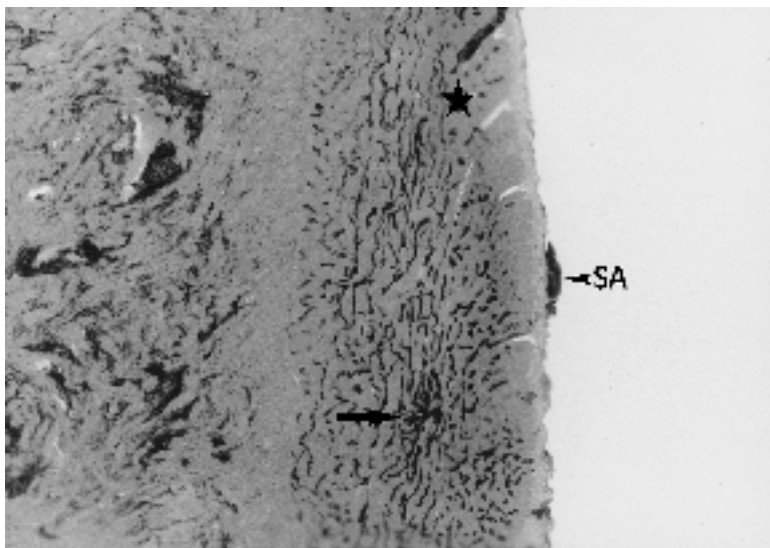
#### Ventricular myoarchitecture

The ventricular myocardium of *Piaractus mesopotamicus*, *Colossoma macropomum* and *Clarias gariepinus* consisted of two distinct layers, an outer compact layer and an inner spongy layer (Figs 8–10).

In the outer compact layer the fibres were arranged in a more compact form and involved the entire ventricle. In *P. mesopotamicus* this layer was quite thick and significantly different on the borders and apex of the



**Fig. 4** Schematic diagram of the distribution of the superficial coronary arteries in the ventricle of teleost fishes: venous sinus (S); atrium (A); ventricle (V); bulbus arteriosus (B); right coronary artery (arrowhead); left coronary artery (→). Ventral view of the heart of *P. mesopotamicus* (a), *C. macropomum* (c) and *C. gariepinus* (e); dorsal view of the heart of *P. mesopotamicus* (b), *C. macropomum* (d) and *C. gariepinus* (f).

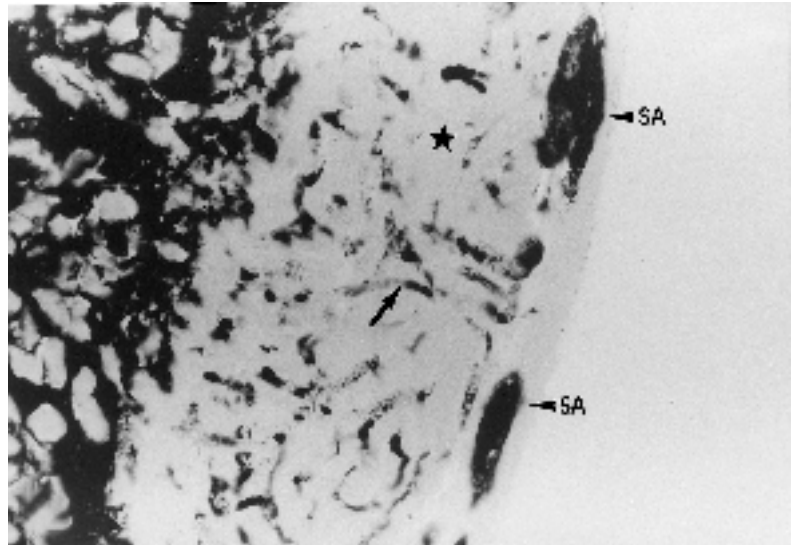


**Fig. 5** Compact layer (\*) of the heart of *P. mesopotamicus* presenting a subepicardial coronary artery (SA) marked with India ink, giving origin to numerous arterial ramifications (→) (×100).

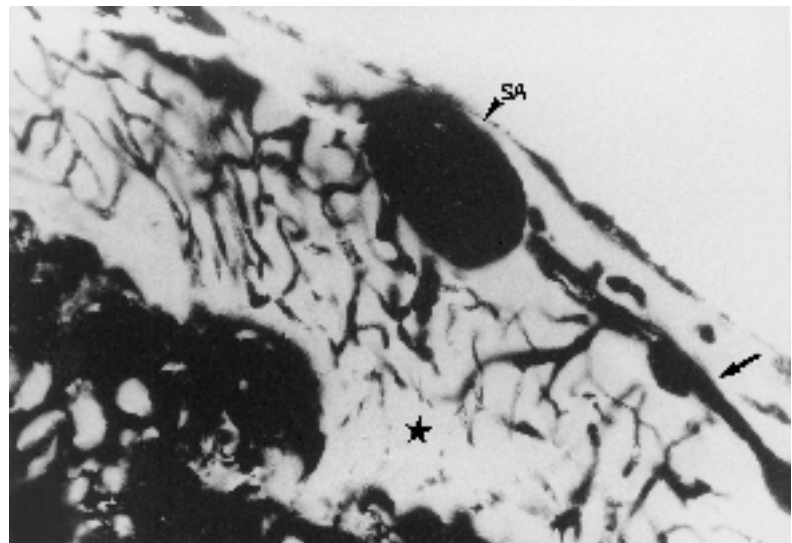
ventricle (Table 1). This layer consisted of two distinct sublayers according to the arrangement of the fibres, i.e. an external layer with a longitudinal arrangement and an internal layer with a circular arrangement (Fig. 8).

In *C. macropomum* the compact layer was of variable thickness, being thicker at the borders than at the ventricular apex (Table 1). Its fibres presented mainly a longitudinal arrangement (Fig. 9). African catfish possess

**Fig. 6** Compact layer (\*) of the heart of *C. macropomum* presenting subepicardial coronary arteries (SA) giving origin to arterial ramifications (→) (×200).



**Fig. 7** High-magnification view of the arterial ramifications (\*) of subepicardial coronary artery (SA) of the compact layer (→) of *C. gariepinus* (×200).



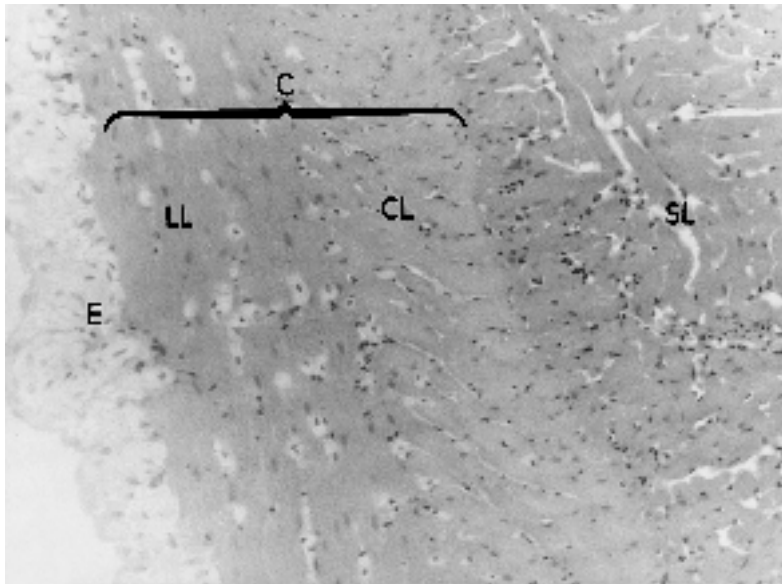
a thin compact layer (Fig. 10), with no significant difference of thickness between apex and ventricular border (Table 1), and longitudinally arranged fibres (Fig. 10). In all species, the fibres of the outer compact layer were a continuation of those of the inner spongy layer. Subepicardial arteries were seen between the epicardium and the compact layer (Figs 5–7), and coronary arteries occurred among the muscle fibres of the compact layer (Figs 5–7 and 9).

The spongy layer was well developed in the three species, consisting of a group of trabeculae. Lacunae of variable sizes and shapes were observed among the fibre bundles, which were larger in *C. gariepinus* since the muscle fibres of this species were widely spaced. A poorly defined arrangement of the spongy fibres was noted, with the fibres arranged in longitudinal, transverse

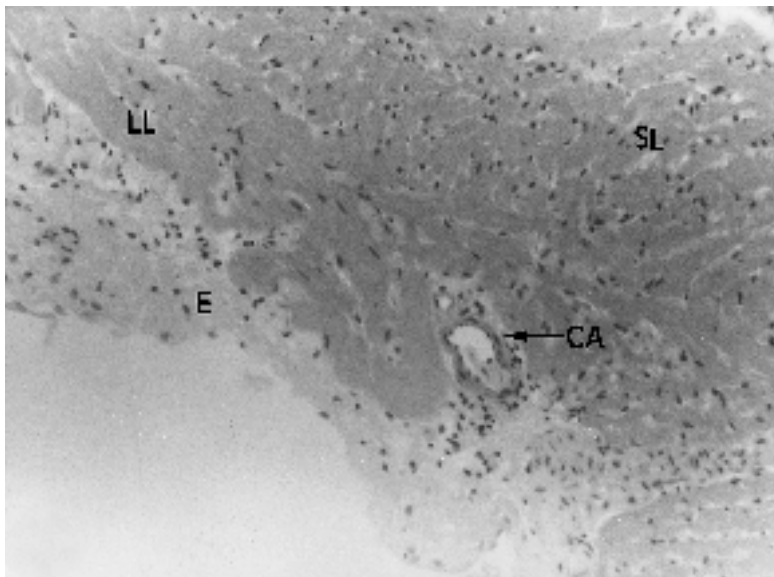
and oblique directions, allowing communication between the ventricular lumen and peripheral areas of the ventricle (Fig. 11).

## Discussion

The cardiac ventricle of teleost fishes presents three distinct shapes characterized as tubular, saccular and pyramidal, which are associated with two structurally different types of myocardium, mixed and spongy (trabecular) (Santer et al. 1983). Earlier studies (Greer Walker et al. 1985; Santer, 1985; Sánchez-Quintana et al. 1995, 1996) have described the saccular and tubular cardiac ventricles, both exclusively consisting of spongy myocardium, which were found in species without active swimming habits, while the pyramidal



**Fig. 8** Detail of the compact layer (C) of the ventricle of *P. mesopotamicus* in longitudinal section, showing the longitudinal compact layer (LL), circular compact layer (CL), epicardium (E) and spongy layer (SL) ( $\times 100$ ).



**Fig. 9** Longitudinal section of the ventricular wall of the heart of *C. macropomum*, showing the outer compact layer with its longitudinal fibres (LL), inner spongy layer (SL), epicardium (E) and coronary arteries (CA) located in the compact layer ( $\times 100$ ).

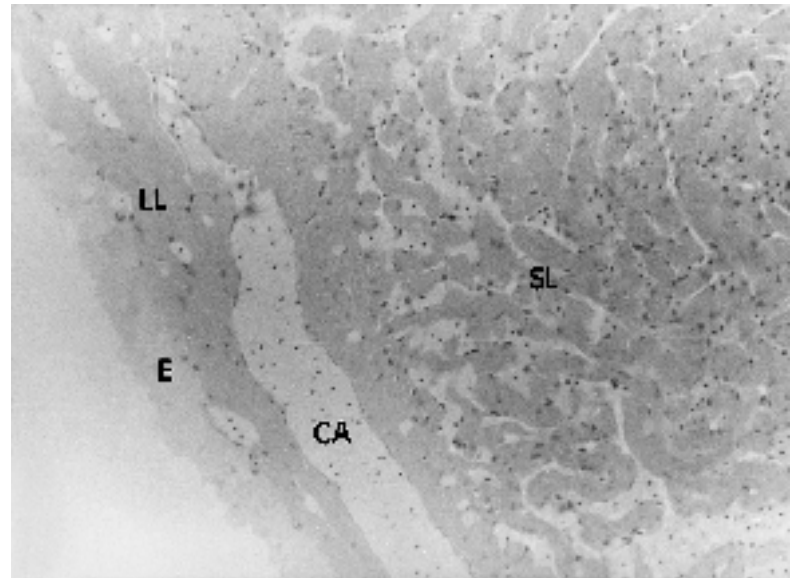
ventricles consisted of mixed myocardium and were found in active species.

In this study, a similar pyramidal cardiac ventricle consisting of an outer compact and inner spongy muscle layers was observed in *P. mesopotamicus* and *C. macropomum*, which have active swimming habits, similar to the cardiac ventricle of some marine species (Santer, 1985; Greer Walker et al. 1985; Sánchez-Quintana et al. 1995, 1996). In contrast to the ventricular structure of other species described in the literature (Greer Walker et al. 1985; Santer, 1985; Sánchez-Quintana et al. 1995, 1996), African catfish, *Clarias gariepinus*, an inactive species, showed a saccular ventricle which also

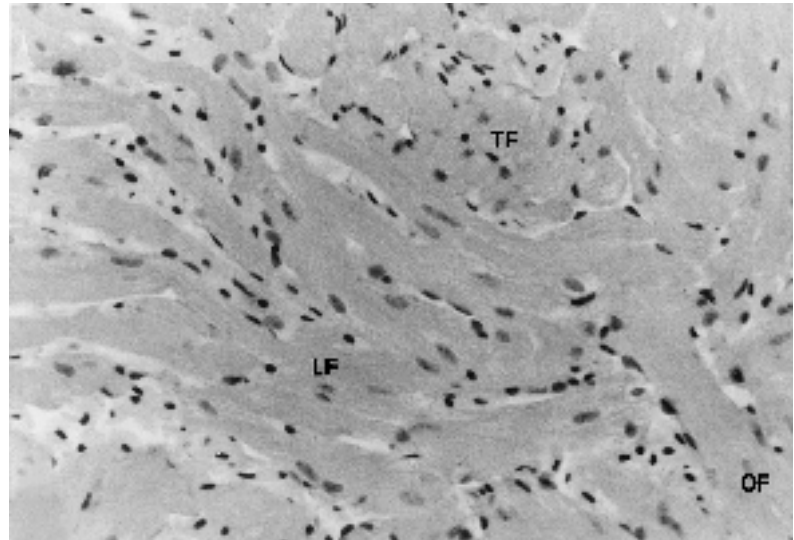
consisted of two muscle layers, an outer compact and an inner spongy layer.

Santer & Greer Walker (1980) described an outer compact myocardial layer in the African catfish species *Clarias lazera*, which lives on the bottom (Nawar, 1955), similar to that observed in *Clarias gariepinus*. Moreover, this structure pattern was observed in hearts of salmon *Oncorhynchus mykiss* (Santer, 1974) and *Salmo salar* (Poupa et al. 1974). These species have a migratory behaviour that requires metabolic adaptation to constant changes in water salinity.

The ventricular myocardium of the three species studied here consisted of outer compact and inner



**Fig. 10** Longitudinal section of the ventricle of *C. gariepinus* showing epicardium (E), a thin outer compact layer containing longitudinal fibres (LL), coronary artery (CA) and inner spongy layer (SL) ( $\times 100$ ).



**Fig. 11** Inner spongy layer of the ventricle of *P. mesopotamicus* in longitudinal section, with its longitudinal (LF), transverse (TF) and oblique fibres (OF) ( $\times 200$ ).

spongy layers. In *C. macropomum*, the muscle fibres were arranged longitudinally in the compact layer, while in *P. mesopotamicus* the compact layer could be subdivided into two layers according to the arrangement of the fibres, i.e. an external longitudinal layer and an internal circular layer of variable thickness. According to Sánchez-Quintana et al. (1996), the compact layer of teleosts consisted of fascicles of cardiac muscle with a predominantly longitudinal arrangement, while in elasmobranchs the myofibres were arranged transversely. These different patterns of cardiac fibre arrangement permitted a reduction in the longitudinal heart diameter in teleosts and in the transverse cardiac diameter in elasmobranchs during ventricular systole.

**Table 1** Median ( $\pm$  SEM) compact layer thickness ( $\mu\text{m}$ ) in the ventricle of the heart

	<i>Piaractus mesopotamicus</i>	<i>Colossoma macropomum</i>	<i>Clarias gariepinus</i>
Ventricular apex	412.0 $\pm$ 2.7	257.9 $\pm$ 2.3	150.7 $\pm$ 1.8
Ventricular border	498.1 $\pm$ 1.4*	334.9 $\pm$ 1.4*	143.4 $\pm$ 1.8

\* $P > 0.0001$ , showing significant difference between means of the apex and ventricular border of each species.

Furthermore, as a consequence of swimming habit, significant differences in the thickness of the cardiac wall at the ventricular borders and cardiac apex were observed in *P. mesopotamicus* and *C. macropomum*, a probable physiological adaptation of the cardiac

architecture to the free swimming condition. The thickness of cardiac ventricle in fishes is also related to establishment of the coronary vascularization of the cardiac wall and other ecophysiological factors (Tota et al. 1983).

African catfish, *Clarias gariepinus*, possessed a thin outer compact layer of similar thickness in the apex and ventricular border in which muscle fibre bundles were arranged longitudinally, with large lacunae observed in the inner spongy myocardium. At greater water depths, such as at the bottom of lakes and rivers, where *C. gariepinus* lives, the pressure is high and the amount of dissolved oxygen is low. Therefore, a myocardium predominantly formed by fine trabeculae would be vital, guaranteeing adequate gas exchange between the myocardium and the circulating oxygen-poor blood (Santer & Greer Walker, 1980), and consequently allowing better oxygenation of the tissues.

In *P. mesopotamicus*, *C. macropomum* and *C. gariepinus*, the myocardium was mainly of the spongy type and was bathed by blood which, in addition to being present in the ventricular lumen, filled the various smaller compartments formed between the myofibres arranged in trabeculae of the heart walls. The muscle fibres showed longitudinal, transverse and oblique arrangements in such way that resistance was minimal in order to permit filling and distension of the ventricle. In this respect, a mainly spongy ventricle consisting of a random arrangement of fibres would favour the diffusion of oxygen to the ventricular lumen in the direction of the outer compact myocardium (Davie & Farrell, 1991). We can confirm this hypothesis on the basis of our results from the three fish species studied.

With regard to cardiac vascularization, the patterns of origin and distribution of the coronary arteries diverge according to the different taxonomic groups (Andrés et al. 1990). In *P. mesopotamicus* and *C. macropomum*, the right and left coronary arteries were observed descending along the lateral surface of the bulbus arteriosus and branching on the heart surface, similar to that seen in some shark species (Grant & Regnier, 1926; Andrés et al. 1990), but this vascular pattern was not a characteristic of *C. gariepinus*.

The typical coronary artery of the heart of ganoid fishes and other teleosts (Grant & Regnier, 1926), such as *C. gariepinus*, originates as a single artery from the fourth branchial arch and reaches the heart through the dorsal surface of the bulbus arteriosus. At its origin, the coronary artery is divided into ventral and dorsal

branches supplying the corresponding surfaces of the heart, as described above.

With respect to the vascularization of the ventricular myocardium of elasmobranchs and teleost fish, Tota (1983) pointed out the presence of a hypobranchial arterial system consisting of hilar and extrahilar systems. According to this author, the hilar system is formed by coronary arteries that deeply penetrate the ventricular wall in the regions of junction between the bulbus and the ventricle. The extrahilar system consists of extramural (subepicardial) vessels and intramural (intramyocardial) vessels that penetrate the ventricular muscle and branch out. In *Piaractus mesopotamicus*, *Colossoma macropomum* and *Clarias gariepinus* the extrahilar system is well developed and consists of coronary arteries that give rise to subepicardial arteries branching out extensively throughout the compact layer.

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