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Morphological and cytochemical characterization of the peripheral blood cells of farmed streaked prochilod *Prochilodus lineatus* (Characiformes, Prochilodontidae)

[*Caracterização morfológica e citoquímica das células do sangue periférico do curimatã* Prochilodus lineatus *(Characiformes, Prochilodontidae) em cultivo*]

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ABSTRACT

Morphological and cytochemical studies of peripheral blood cells of fish have improved the understanding of their functions and cell types. The present study performed the Morphological and cytochemical analysis of the peripheral blood of *Prochilodus lineatus*, Characiform native to South America, which has been gaining space in local aquaculture and as a species introduced in Asia. Our analysis provided information on the morphological and cytochemical characteristics of the leukocytes, for the formulation of hypothesis about their role in the immune system of the species. It was found that *Prochilodus lineatus* has morphological and cytochemical features in common with other fish species, mainly of the Characiformes order. However, we detected the presence of heterophils and PAS positive granulocytes simultaneously with neutrophils. We also found that heterophils and PAS positive granulocytes are very similar, both morphologically and cytochemically.

Keywords: Cytochemistry, hematology, *Prochilodus lineatus*

RESUMO

O estudo da morfologia e da citoquímica das células do sangue periférico dos peixes tem sido eficaz para o entendimento de suas funções e dos tipos celulares. Este estudo realizou a análise morfológica e citoquímica do sangue periférico de Prochilodus lineatus*, caracídeo nativo da América do Sul que vem ganhando espaço na aquicultura local e como espécie introduzida na Ásia. Essa análise forneceu informações sobre a morfologia e as características citoquímicas dos leucócitos, visando a hipóteses sobre suas funções. Verificou-se que estas são semelhantes em vários aspectos a outras espécies, principalmente da ordem Characiformes. No entanto, neste estudo detectou-se a presença dos heterofilos e da célula granulocítica especial, simultaneamente à presença dos neutrófilos. Ainda, foi verificado que os heterofilos e a célula granulocítica especial são muito semelhantes morfológica e citoquimicamente.*

Palavras-chave: citoquímica, hematologia, Prochilodus lineatus

INTRODUCTION

Fish leukocytes are a very heterogeneous group, and there are differences even among closely related species. These cells are less differentiated in comparison to their mammal counterparts, making their identification more difficult (Shigdar *et al*., 2009). Another aspect that complicates the identification of leukocyte subpopulations in fish is the fact that peripheral blood is the site of maturation and differentiation of these cells. Therefore, cells in different stages of maturation

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can be observed, which vary in their morphology and enzymatic profile (Zhang *et al*., 2018). Although fish leukocytes have been classified according to the same criteria and terminology applied to mammals, given the morphological and functional similarity between them (Palic *et al.*, 2011), traditional methods of identification with Romanovsky dyes have not been entirely reliable for this purpose (Tavares Dias, 2006). For a better characterization of these cells and elucidation of their role in fish, techniques that demonstrate their enzymatic profile and/or structural components are necessary, since they differ among leukocyte subpopulations and maturation stages. In this regard, cytochemical staining and morphological analysis can provide additional information that enables the identification of leukocyte subpopulations and their likely functions (Shigdar *et al*., 2009).

Thus, the aim of the present study was characterizing cytochemically and morphologically the aspects of peripheral blood cells of the streaked prochilod (*Prochilodus lineatus*), a South American native characid that has been gaining ground in Brazilian aquaculture with other native species (Boscolo *et al*., 2011) and was introduced in Asian aquaculture (China and Vietnam) (Kalous *et al*., 2011).

MATERIAL AND METHODS

The present study was approved by the Animal Use Ethics Committee of the Federal Fluminense University (protocol number 888) and by the Animal Use Ethics Committee of the Fisheries Institute of Rio de Janeiro State (protocol number 08/2017).

Thirty juveniles of streaked prochilod (*Prochilodus lineatus*) (average size (total length) 18.27±0.73cm, average weight 66.41±6.25g) from a fish farm of the city of Cordeiro, State of Rio de Janeiro, Brazil, were reared in pond and fed twice a day, at 9am and 4pm with a commercially formulated diet containing 400g/kg protein, 350mg/kg vitamin C, 80g/kg ethereal extract and 100g/kg moisture (levels and guarantees made available by the manufacturer). Fish feed was suspended for a 24-hour period before the sampling procedure. The specimens were captured with a net and anesthetized with Eugenol solution (50mg/L) (Medeiros Junior *et al.*, 2019) until loss of balance and swimming ability were observed. Then, peripheral blood was taken from the caudal veins with a 20 X 0.55mm needle and 3mL syringe. After sampling, the blood was transferred to microtainer tubes (0.5mL) containing EDTA. A drop of blood sample was spread to produce smears for cytochemistry and morphological analysis. Water quality was monitored during the experimental period, with average temperature of 23.7°±2.8°C; average dissolved and saturated oxygen at 5.7±1.3mg/L and 77.2±12.7%, respectively; average pH at 8.3±0.4; average electric conductivity at 0.15±0.01µS/cm; average total dissolved solids at 0.08±0.01ppt, and average total ammonia at 0.41±0.13mg/L. Physical and chemical monitoring of water was carried out using a HI83203-01 Hanna photometer, HI9146-04 Hanna oxygen meter and a HI98130 Hanna multi-parameter combo.

For the cytochemical study, the following procedures were adopted: The PAS stain was performed accordingly to Zhang *et al*. (2018), in which the dried blood smears were covered with periodic acid (Merck Milipore®) 0.5% for 5 minutes and rinsed with distilled water. Then, the smears were covered with Schiff's stain (Merck Milipore®) for 10 minutes and washed under running water for 5 minutes. After, the smears were re-stained with haematoxylin (EasyPath®) for 3 minutes.

The Sudan Black B (SBB) stain was performed accordingly to Ranzani Paiva *et al*. (2013), in which the dried blood smears were fixed with ethanol 70% for 5 seconds and immersed in SBB stain (Dinâmica®) for 60 minutes. The smears were then rinsed in ethanol 70% and washed in running water for 5 minutes, followed by re-stain with haematoxylin (EasyPath®) for 3 minutes.

The peroxidase detection was performed accordingly to Jain (1986), in which the dry blood smears were covered with benzidine solution (100mL of distilled water, 1g of benzidine (Sigma Aldrich®) and 3 drops of hydrogen peroxide 3% (Farmax®)) for 30 seconds, covered with distilled water for 2 minutes and washed in running water for 5 minutes. Then, the smears were re-stained with Giemsa (Merck Milipore®) for 30 minutes.

The slides were observed through a Leica DM750 microscope and photographed with a Leica ICC50

camera. The photos were analyzed and processed in the open-source image processing package Fiji.

Blood smears for morphological analysis of peripheral blood cells were stained with Giemsa (Merck Milipore®) for 30 minutes, examined under the Leica DM750 microscope and photographed with the Leica ICC50 camera. The photos were analyzed and processed in the open-source image processing package Fiji.

RESULTS

Other studies that examined the peripheral blood cells of *Prochilodus lineatus* obtained varied results concerning the leukocyte types. While some cite the presence of lymphocytes, neutrophils, monocytes and eosinophils (Tavares Dias *et al.*, 2008; Cazenave *et al.*, 2009; Belo *et al.*, 2013; Tavares Dias, 2015), another study reports the presence of these cells and the PAS positive granulocyte (Ranzani Paiva *et al.*, 1999). The present study found all the leukocytes cited in those studies, in addition to heterophils.

Lymphocytes (Fig. 1) are predominantly round, but they can be occasionally elongated or amorphous, with scarce basophilic cytoplasm and nucleus purple or violet when stained with Giemsa, with a round, elongated or chamfered shape. The nucleus has dense chromatin and the cell has a high nucleus: cytoplasmic ratio. The cytoplasm can also extend into small projections (blebs).

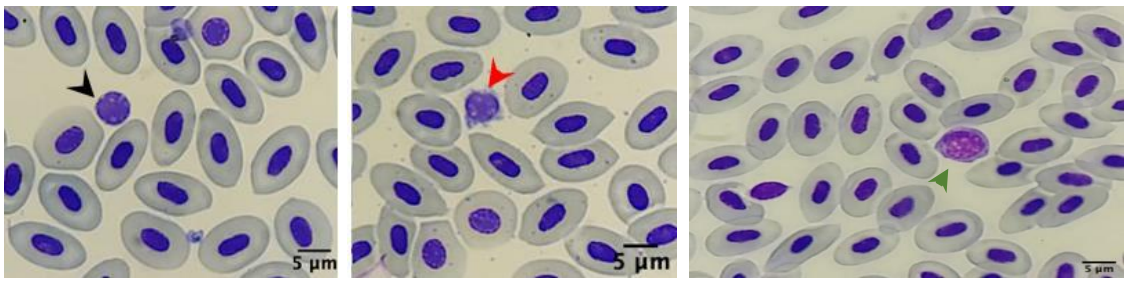

Figure 1. Lymphocytes of *Prochilodus lineatus* stained with Giemsa. Black arrowhead – lymphocyte showing scarce cytoplasm; red arrowhead – lymphocyte showing cytoplasmatic projections (blebs); green arrowhead - lymphocyte showing basophilic cytoplasm. 100X magnification.

Monocytes (Fig. 2) are large agranulocytes, round shaped or amorphous (possibly, according to the activation stage). Stained with Giemsa, the abundant cytoplasm is intensely basophilic and usually contains vacuoles and projections. The nucleus is eccentric and eosinophilic, with loose chromatin, and its shape usually follows the cell shape.

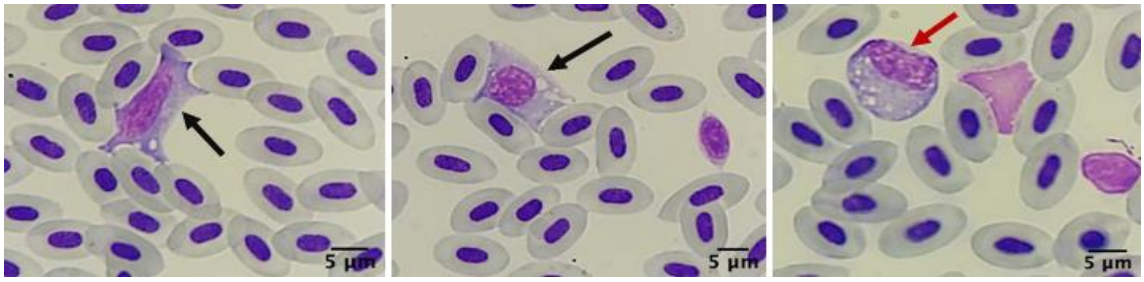

Figure 2. Monocytes of *Prochilodus lineatus* stained with Giemsa. Black arrows – amorphous monocytes showing cytoplasmatic projections and vacuoles; red arrow – round shaped monocyte, with abundant basophilic cytoplasm containing vacuoles, and eccentric eosinophilic nucleus. 100X magnification.

Neutrophils (Fig. 3) are the granulocytes most seen in the species. They are cells of variable sizes and shapes (round, elongated or amorphous), with clear to grayish cytoplasm where a fine granulation can be seen, and which can also have vacuoles. The nucleus can have different shapes (round, chamfered, horseshoe shaped, elongated, segmented) and loose chromatin, and stained with Giemsa, it has a purple to violet hue. Some forms (possibly non-activated or immature forms) are

usually round and medium sized cells, with a round or horseshoe shaped nucleus and gray cytoplasm. It can also be seen as elongated cells with cytoplasmatic projections, clearer cytoplasm and the nucleus shape also follows the cell shape (possibly the activated form).

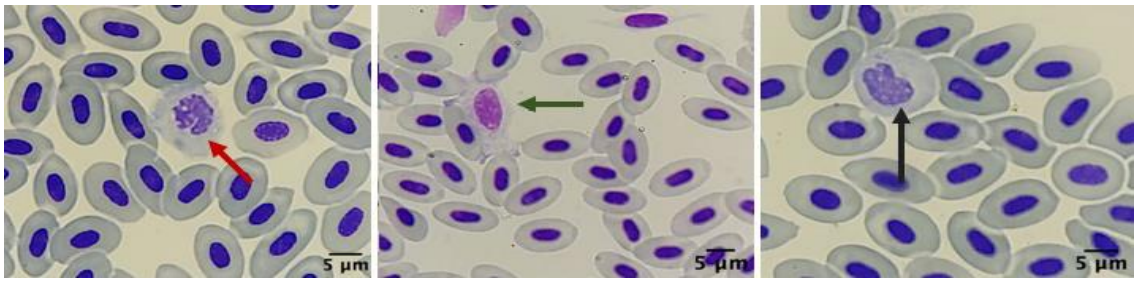

Figure 3. Neutrophils of *Prochilodus lineatus* stained with Giemsa. Red arrow – round shaped neutrophil showing horseshoe shaped nucleus and gray cytoplasm; green arrow – elongated neutrophil with cytoplasmatic projections; black arrow – round neutrophil with chamfered nucleus and gray cytoplasm. 100X magnification.

Heterophils (Fig. 4) are medium sized to large cells, round or elongated, with a round to oval eccentric nucleus with granular eosinophilic cytoplasm. The cytoplasm is abundant, clear and filled with small granules, rod or oval shaped, which has variable dyeing characteristics (basophilic and eosinophilic) when stained with Giemsa. It can be also seen as a smaller cell, round shaped, with scarce and eosinophilic cytoplasm (possibly, an immature or non-activated form of heterophil).

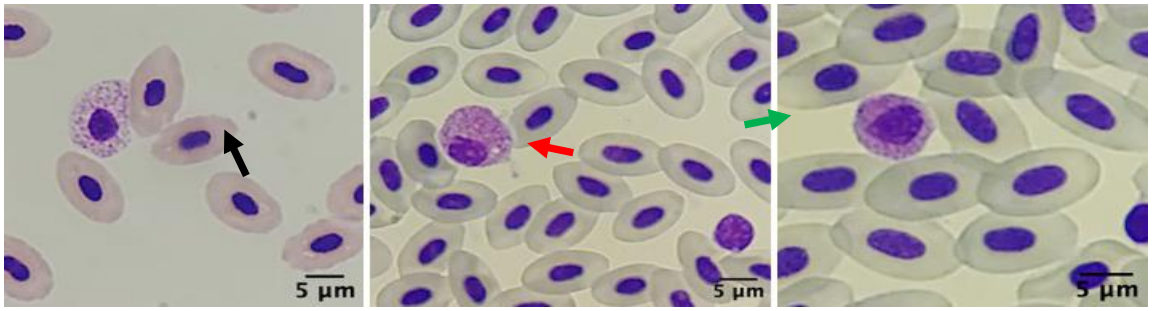

Figure 4. Heterophils of *Prochilodus lineatus* stained with Giemsa. Black arrow – heterophil with clear cytoplasm filled with granules of variable stain characteristics; red arrow – round heterophil with eccentric eosinophilic nucleus and abundant cytoplasm filled with granules of variable stain characteristic; green arrow – heterophil with scarce eosinophilic cytoplasm filled with granules. 100X magnification.

The PAS positive granulocyte (Fig. 5) is a small round cell, with basophilic nucleus and dense chromatin. The cytoplasm is slightly basophilic and contains granules that are not susceptible to staining with acidic or basic dyes, appearing as a fine granulation. Some of these cells have a clearer cytoplasm and more evident granulation.

Eosinophils (Fig. 6) are small granulocytes, with eccentric and eosinophilic round to oval nucleus; the cytoplasm is clear, containing large and coarse eosinophilic granules, usually round or rod shaped. Some forms, possibly representing the immature eosinophil, have scarce cytoplasm and central nucleus.

Thrombocytes (Fig. 7) are small cells with variable shapes, which can be round, elongated or fusiform, depending on the activation stage. Activated forms are fusiform and have spicules or projections in their membranes. Non-activated cells usually have a round shape. Stained with Giemsa, the scarce cytoplasm is clear or reddish, with a basophilic nucleus that occupies nearly the entire cell; the nucleus shape follows the cells shape.

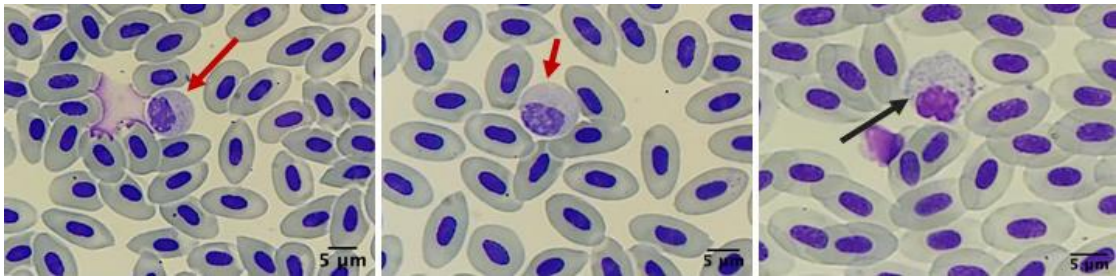

Figure 5. PAS positive granulocyte of *Prochilodus lineatus* stained with Giemsa (red arrows). Black arrow – PAS positive granulocyte showing clearer cytoplasm, with more evident granulation. 100X magnification.

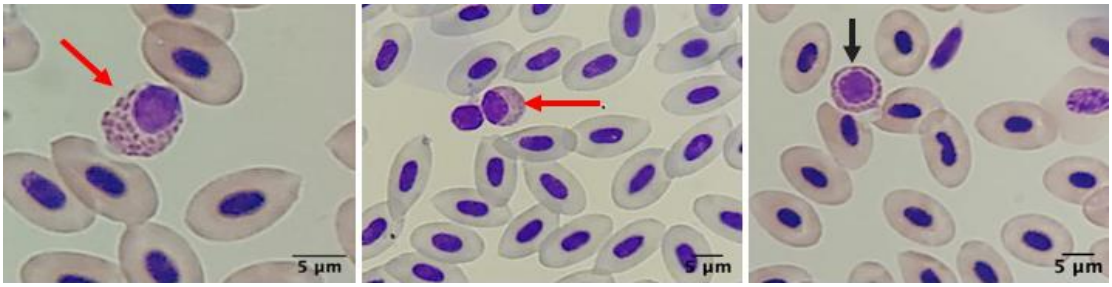

Figure 6. Eosinophils of *Prochilodus lineatus* stained with Giemsa (Red arrows). Black arrow – eosinophil with scarce cytoplasm containing eosinophilic granulation and central nucleus. 100X magnification.

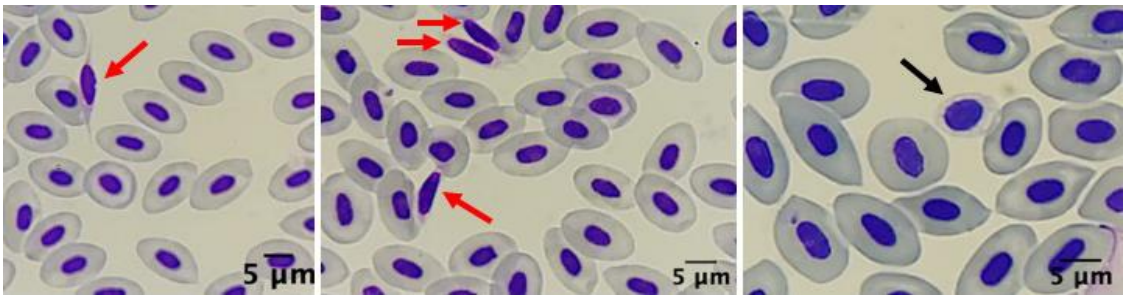

Figure 7. Thrombocytes of *Prochilodus lineatus* stained with Giemsa. Red arrow – activated thrombocytes, showing fusiform shape with cytoplasmatic spicules. Black arrow – non-activated thrombocyte, with round shape and clear scarce cytoplasm. 100X magnification.

The present study used two non-enzymatic reactions – periodic acid Schiff (PAS) to detect glycogen and Sudan Black B (SBB) to detect phospholipids – and one enzymatic reaction – benzidine to detect peroxidase. The results are summarized in Tab. 1 and Fig. 8.

The positive reaction to PAS is characterized by a magenta coloration in the cell cytoplasm, which can vary in intensity (light red to intense magenta). Cells positive for SBB have grey cytoplasmatic granules, while cells positive for peroxidase have a diffuse grey coloring in the cytoplasm, which can vary in intensity (light grey to dark grey). Neutrophils show positive reactions to all cytochemical stains used, which can vary in intensity (positive to strongly positive). Lymphocytes, monocytes and eosinophils have negative reactions to all cytochemical stains used, while heterophils have a negative reaction only to peroxidase; for PAS, the reaction is strongly positive. PAS positive granulocyte has identical reactions to those of the heterophils – strongly positive for PAS, positive for SBB and negative for peroxidase. Some thrombocytes have positive reactions only for PAS, showing positive spots in the cytoplasm. Not all thrombocytes had this reaction.

Table 1. Cytochemistry of peripheral blood cells of *Prochilodus lineatus*

	Lymphocyte	Neutrophil	Heterophil	Monocyte	Eosinophil	PAS+	Thrombocyte
PAS	-	+ / +++	+++	-	-	+++	+ (focal) /-
PER	-	+ / +++	-	-	-	-	-
SBB	-	+ / +++	+	-	-	+	-

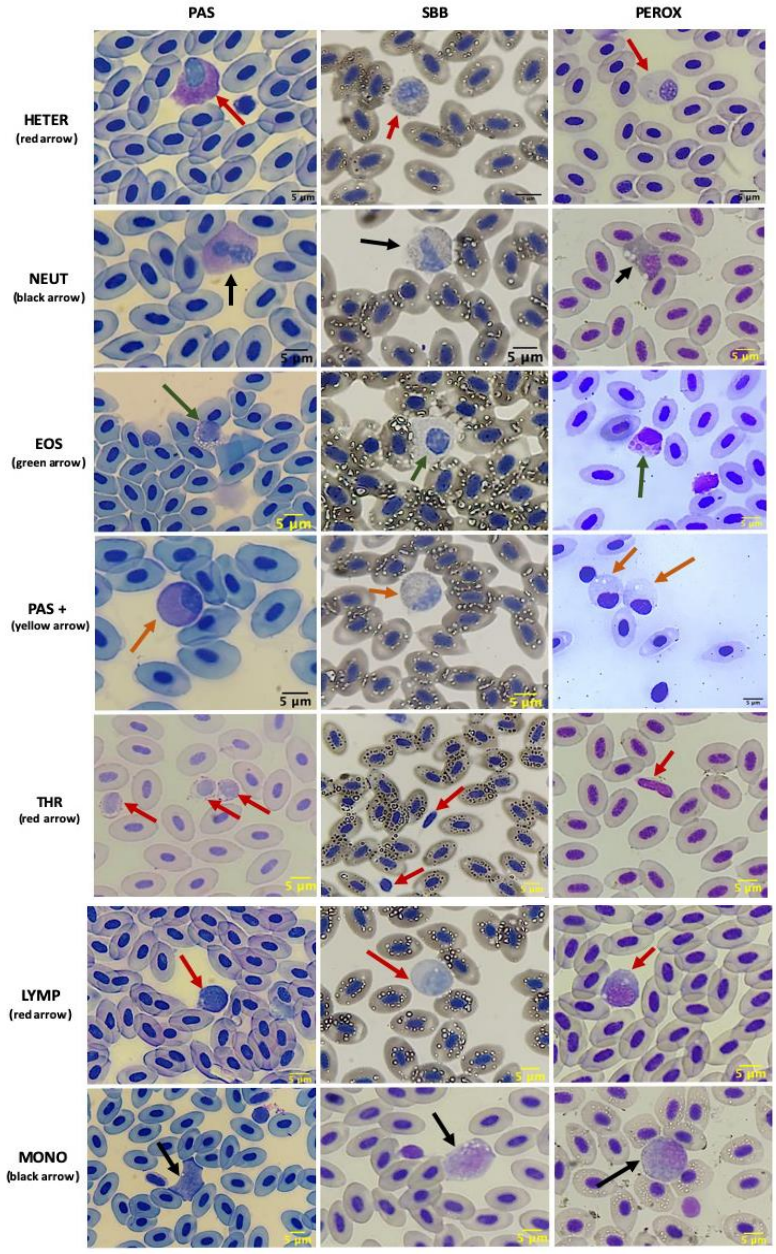

Figure 8. Cytochemistry of peripheral blood cells of *Prochilodus lineatus*. PAS = Periodic acid Schiff stain; SBB = Sudan Black B stain; PEROX = Peroxidase stain; HETER = heterophils; NEUT = neutrophils; EOS = eosinophils; PAS + = PAS positive granulocyte; THR = thrombocytes; LYMP = lymphocytes; MONO = monocytes. 100X magnification.

DISCUSSION

The morphology of *Prochilodus lineatus* blood cells in the present study is consistent with the observation of Tavares Dias (2015) regarding the same species. However, this author did not report the presence of heterophils nor the PAS positive granulocytes in this study. Concerning other species of the Characiformes order, studies with peripheral blood cells of the species *Brycon orbignyanus* (Tavares Dias; Moraes, 2006), *Salminus maxillosus* (Veiga *et al*., 2000) and *Salminus brasiliensis* (Pádua *et al*., 2009) reported the simultaneous presence of neutrophils and heterophils, as it was found in the present study. The morphology of peripheral blood cells in *Piaractus mesopotamicus* (Tavares Dias *et al*., 1999a), *Colossoma macropomum* (Tavares Dias *et al*., 1999b), *Astianax bimaculatus* and *Hoplias malabaricus* (Tavares Dias, 2006) is similar to what was observed in *Prochilodus lineatus* in the present study. Nonetheless, the presence of heterophils was not reported in these species. PAS positive granulocytes have been reported in Characiformes species – *Piaractus mesopotamicus, Colossoma macropomum* (Tavares Dias *et al*., 1999a, 1999b, 2003; Tavares Dias, 2015) and in Cypriniformes species *Aristichthys nobilis* (Tavares Dias, 2006) and *Cyprinus carpio* (Tavares Dias *et al.*, 2003; Cazenave *et al.*, 2009), whose morphology is similar to what has been reported in *Prochilodus lineatus* in the present study. In *Cyprinus carpio*, the study conducted by Tripathi *et al*. (2004) classified this cell as basophil, but its morphology is very alike to the PAS positive granulocyte, and have similar cytochemical reactions (positive for PAS, negative for PER). The study of Tavares Dias (2015) with *Cyprinus carpio* classified it as PAS positive granulocyte, and in his study with *Aristichthys nobilis* (Tavares Dias *et al.*, 2003) the author suggests that this cell can be a precursor of the basophil, though without the characteristic metachromasia. In a subsequent study (Tavares Dias, 2006), the same author established the impossibility of a PAS positive granulocyte being a precursory cell due to its strong positive reaction to PAS, since immature cells do not show such intense reaction because of the lower glycogen content in its granules. In the present study, PAS positive granulocytes showed morphological and cytochemical similarities with heterophils, which may indicate that PAS positive granulocytes and heterophils may be cells from the same lineage in *Prochilodus lineatus*.

In mammals, the presence of peroxidase in primary lysosomal granules is shown from the immature stages of neutrophils (Raskin, 2010). This enzyme produces superoxide radicals and halogen ions inside the phagosomes, forming an efficient system against bacterial infections. Therefore, the presence of peroxidase is a strong sign of antibacterial and phagocytic function (Shigdar *et al*., 2009; Silva *et al*., 2011), making this enzyme a good marker for leukocyte activation in superior vertebrates (Rodrigues *et al*., 2003). In fishes, a positive reaction to peroxidase is mostly seen in neutrophils, and this reaction is more evident as the cell matures due to accumulation of the enzyme in its granules (Ranzani Paiva *et al*., 2013). Thus, the intensity of the reaction to peroxidase in fish neutrophils can demonstrate its stage of maturation and activation and indicates a possible phagocytic and antibacterial function. Such variation in the intensity of the reaction was observed in neutrophils of *Prochilodus lineatus*, demonstrating the presence of these granulocytes in various stages of maturation and activation in the peripheral blood of the species.

Periodic acid Schiff shows glycoproteins, mucoproteins, glycolipids and high molecular weight carbohydrates; in blood cells, PAS highlights the presence of glycogen (Tavares Dias; Moraes, 2006). Glycogen in these cells is related to phagocytosis, whose mechanism is energy dependent (Ueda *et al*., 2001). Although mature neutrophils are metabolically inactive in the blood stream, when mobilized they experience a rapid increase in their activity and need an energy source rapidly available. Besides, when they migrate to the tissues, they enter an environment with low levels of oxygen and glucose, which require a ready to use source of energy, even in anaerobic conditions (Tavares Dias, 2006). Therefore, the presence of glycogen in leukocytes may indicate phagocytic activity, and the intensity of reaction to PAS increases with cell maturation due to accumulation of the polysaccharide (Fang *et al*., 2014).

Lipids inside the granulocytes, including phospholipids, neutral fatty acids and steroids, are identified by the Sudan Black B stain. In neutrophils, secondary granules have positive

staining, while primary granules also have a positive, though weaker reaction. Therefore, SBB shows all stages of neutrophil maturation by the intensity of its reaction – cells in the final stage of differentiation show intense reaction, while immature cells demonstrate a weak reaction (Raskin *et al*., 2010; Ranzani Paiva *et al*., 2013).

The cytochemical characteristics of neutrophils of *Prochilodus lineatus* (positive for glycogen, peroxidase and SBB) indicate that this cell can be the main phagocytic granulocyte of this species and are consistent with other species of the Characiformes order – *Salminus maxilosus* (Veiga *et al.,* 2000), *Brycon amazonicus* (Santos *et al.*, 2011), *Brycon orbignyanus* (Tavares Dias; Moraes, 2006), *Astianax bimaculatus* and *Hoplias malabaricus* (Tavares Dias, 2006). Ranzani Paiva *et al.* (2013) reported a positive reaction to PAS and negative reaction for peroxidase in *Prochilodus lineatus* neutrophils. *Colossoma macropomum,* also a Characiform, differs in the negative reaction to SBB (Salazar Lugo *et al.*, 2012), indicating that there are differences in the constituents of intracellular membranes in neutrophils (Ueda *et al*., 2001) even between related species. In species of other taxonomic categories, like Cypriniformes (*Cyprinus carpio* (Tripathi *et al*., 2004), *Carassius auratus*, *Ctenopharyngodon idellus* (Zhang *et al*., 2018), *Schizothorax prenanti* (Fang *et al*., 2014), *Gymnocypris eckloni* (Zheng *et al*., 2017) and *Aristichthys nobilis* (Tavares Dias, 2006)), Siluriformes (*Sorubim lima* (Bianchi *et al*., 2014), *Hoplosternum litoralle* (Tavares Dias and Barcellos, 2005)) and Perciformes (*Centropomus parallelus* (Silva *et al*., 2011), *Maccullochella peelii* (Shigdar *et al*., 2009), *Oreochromis niloticus* (Ueda *et al*., 2001) and *Astronotus ocellatus* (Tavares Dias, 2006)), the cytochemical reactions are similar to those of *Prochilodus lineatus* in the present study, indicating the phagocytic role of this granulocyte in teleosts in general.

The heterophils found in other species of the Characiformes order show variable cytochemical characteristics, mainly in reaction to peroxidase. As for the latter, unlike what was found for *Prochilodus lineatus* in this study, it is positive in *Salminus maxillosus* (Veiga *et al*., 2000) and *Brycon amazonicus* (Santos *et al*., 2011); in *Brycon orbignyanus* (Tavares Dias and Moraes, 2006), this reaction is negative. Such difference can be explained by the possibility that most granules of heterophils of *Prochilodus lineatus* and *Brycon orbignyanus* are specific, and these granules are devoid of peroxidase (Ranzani Paiva *et al*., 2013). The reaction to PAS is positive in these species (Veiga *et al*., 2000; Tavares Dias; Moraes, 2006), as it was found in this study. A previous cytochemical study of *Prochilodus lineatus* blood cells (Ranzani Paiva *et al*., 2013) did not refer to the presence of heterophils. In other taxonomic categories investigated, heterophils were observed only in Siluriformes *Hoplosternum litoralle* (Tavares Dias and Barcellos, 2005), and it also showed a positive reaction to PAS, which may indicate phagocytic function of this cell in different taxonomic categories. Concerning SBB, *Salminus maxillosus* showed a negative reaction (Veiga *et al*., 2000) and *Brycon amazonicus* (Santos *et al*., 2011) showed a positive reaction, as was observed in the present study. This may suggest that there are differences in the constituents of intracellular membranes also in heterophils.

The eosinophils of *Prochilodus lineatus* observed in this study showed cytochemical reactions identical to what was found for Characiformes *Salminus maxillosus* (Veiga *et al*., 2000) (negative for PAS, SBB and peroxidase). Ranzani Paiva *et al*. (2013) also reported a negative reaction of *Prochilodus lineatus* eosinophils to PAS and peroxidase. This negative reaction may indicate a non-phagocitic function at the immune defense of these species. In other taxonomic categories, cytochemical reactions had variable results. Cypriniformes *Cyprinus carpio* (Tripathi *et al*., 2004) had identical reactions to those observed in the present study, while *Gymnocypris eckloni* (Zheng *et al*., 2017) differed in the PAS reaction, which was positive. Among the Siluriformes, *Hoplosternum litoralle* (Tavares Dias and Barcellos, 2005) had reactions similar to *Prochilodus lineatus* in the present study, while *Sorubim lima* differed in the positive reaction to SBB (Tavares Dias and Barcellos, 2005). In Perciformes, *Oreochromis niloticus* (Ueda *et al*., 2001) had positive reactions to peroxidase and SBB, unlike the findings of the present study; *Astronotus ocellatus* (Tavares Dias, 2006) showed a positive reaction only to PAS. These results indicate that the eosinophil may have different roles in different species.

PAS positive granulocyte is characterized by its strong positive reaction to PAS, wich was verified in the present study. This cell was observed in cytochemical studies of *Sorubim lima* (Siluriformes) (Bianchi *et al*., 2014), which differed from the present study in the negative reaction to SBB, and in the Cypriniformes *Aristichthys nobilis* (Tavares Dias, 2006), which showed similar reactions to *Prochilodus lineatus.* A previous cytochemical study of *Prochilodus lineatus* (Ranzani Paiva *et al*., 2013) did not refer the presence of this cell. In the verified species, the reaction to PAS is strongly positive, indicating that this is not an immature or precursory cell, as glycogen detection in these cells is more visible only in mature cells because of the accumulation of this polysaccharide (Tavares Dias *et al*., 2003).

Thrombocytes of *Prochilodus lineatus* showed a positive reaction to PAS, which may suggest a possible phagocytic function. There are reports in the literature about the role of thrombocytes in phagocytosis of foreign bodies (Ueda *et al*., 2001). Ranzani Paiva *et al*. (2013) reported the same cytochemical reactions in thrombocytes of *Prochilodus lineatus* – positive to PAS and negative for peroxidase. The presence of glycogen in the cytoplasm of thrombocytes is common in Characiformes species (Tavares Dias, 2006; Tavares Dias and Moraes, 2006; Santos *et al*., 2011; Fang *et al*., 2014), Siluriformes (Tavares Dias and Barcellos, 2005; Bianchi *et al*., 2014), Perciformes (Ueda *et al*., 2001; Tavares Dias, 2006; Silva *et al*., 2011) and Cypriniformes (Tavares Dias, 2006; Fang *et al*., 2014; Zheng *et al*., 2017; Zhang *et al*., 2018).

The monocytes of *Prochilodus lineatus* observed in the present study showed negative reactions to all cytochemical tests performed, and these reactions were observed in other Characiformes (Tavares Dias, 2006; Tavares Dias and Moraes, 2006; Santos *et al*., 2011; Fang *et al*., 2014). Ranzani Paiva *et al*. (2013) reported similar reactions to PAS and peroxidase in monocytes of *Prochilodus lineatus.* However, *Brycon amazonicus* showed positive reactions to peroxidase and SBB (Santos *et al*., 2011). In other Siluriformes (Tavares Dias and Barcellos, 2005; Bianchi *et al*., 2014) and Perciformes species (Tavares Dias, 2006; Silva *et al*., 2011) monocytes also have the same reactions. In some Cypriniformes species (Fang *et al*., 2014; Zheng *et al*., 2017; Zhang *et al*., 2018) monocytes show positive reactions to PAS. As monocytes are actively phagocytic cells, especially in acute inflammatory responses in tissues (Hrubec and Smith, 2010; Campbell, 2015), the presence of enzymes in these cells is consistent with their function. In the species in which glycogen and peroxidase detection are negative, like *Prochilodus lineatus*, the energy source needed for migration and phagocytosis must be predominantly exogenous, while the hydrolases used in the phagocytosis process possibly belongs to the groups of phosphatases or esterases. According to Ranzani Paiva *et al*. (2013), monocytes of *Prochilodus lineatus* show weak positive reaction to non-specific sterases.

The lymphocytes of *Prochilodus lineatus* observed in the present study showed negative reactions to all cytochemical tests performed, which matches a non-phagocytic and predominantly humoral role reported in the literature (Hrubec and Smith, 2010; Campbell, 2015). Ranzani Paiva *et al*. (2013) reported similar cytochemical reactions. The same reactions are observed in other Characiformes species (Veiga *et al*., 2000; Tavares Dias, 2006; Tavares Dias and Moraes, 2006; Santos *et al*., 2011; Salazar Lugo *et al*., 2012), also in Siluriformes (Tavares Dias and Barcellos, 2005; Bianchi *et al*., 2014) and Perciformes (Tavares Dias, 2006; Silva *et al*., 2011); *Oreochromis niloticus* differ only in the focal positive reaction to PAS in some lymphocytes (Ueda *et al*., 2001). The lymphocytes of some Cypriniformes species show positive reaction to PAS (Fang *et al*., 2014; Zheng *et al.*, 2017; Zhang *et al*., 2018), indicating the need for an endogenous energy source in those leukocytes.

CONCLUSION

In the present study, the morphological and cytochemical analysis of the peripheral blood cells of *Prochilodus lineatus* showed that these cells are similar to the peripheral blood cells of other teleost species, especially those of the Characiformes order. However, we observed the presence of heterophils and PAS positive granulocyte simultaneously with the presence of neutrophils, and furthermore, that heterophils and PAS positive granulocytes are similar, both cytochemically and morphologically, in *Prochilodus lineatus*.

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