



Research article

The kidney structure and hematology of tank-reared-male slender walking catfish (*Clarias nieuhofii* Valenciennes, 1840): From the hematopoietic tissue to the peripheral blood cells

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Abstract

The slender walking catfish *Clarias nieuhofii* is an economically significant species and a potential candidate for aquaculture in Thailand, but its kidney organization and hematology remain undescribed in the literature. The present study aimed to investigate the hematopoietic structure and ultrastructure of the kidney and peripheral blood cells of male *C. nieuhofii* obtained from a breeding site in Thailand. Their kidneys were processed for morpho-histology and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays using transmission electron microscopy. Blood cell smears were stained with the Alcian Blue/Periodic acid-Schiff method. The kidney of *C. nieuhofii* was a paired triangular mass comprising anterior (AK) and posterior (PK) regions characterized by renal corpuscle and hematopoietic tissue. In the AK, only hematopoietic tissue and blood capillaries were identified. Hematopoietic tissue was more prominent in the AK than the PK, and its cells were rarely positive in the TUNEL assay. Melano-macrophage centres were observed in the hematopoietic mass in both regions. The detailed hematopoietic ultrastructure was classified into three series, including erythropoiesis, granulopoiesis and lymphopoiesis, which were shared between both kidney regions. Erythropoiesis and granulopoiesis were evident than lymphopoiesis in the kidney tissue. Erythropoietic differentiation was evident. Proerythroblasts were larger than young erythrocytes. Nucleated erythrocytes were observed in peripheral blood smears. The granulopoietic series involved the development of myeloblasts into granulocytes. White blood cells, especially lymphocytes, were also identified. The present study provides useful insights into the renal adaptive histopathology and physiology of *C. nieuhofii* for aquaculture.

Keywords: Blood cells, Hematopoietic tissue, Kidney, Slender walking catfish, Transmission electron microscopy (TEM).

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INTRODUCTION

The slender walking catfish *Clarias nieuhofii* Valenciennes, 1840 is an economically important freshwater catfish inhabiting peat swamp forests of Southeast Asian countries (Santos et al., 2015). The species is valued as a source of high-quality protein and as an ornamental fish (Muttakin, 2018) but wild populations have declined due to overfishing and habitat degradation (Humphrey and Bain, 1990; Vidthayanon, 2005; Pechsiri and Vanichanon, 2016). Since *C. nieuhofii* is a vulnerable species (Humphrey and Bain, 1990; Vidthayanon, 2005), the development of its aquaculture is of some importance. The literature about *C. nieuhofii* includes studies of its hematology (Sukrakanchana et al., 2012), chromosomes (Saowakoon and Machoo, 2021) and cell lines (Lertworapreecha et al., 2021) but its kidney structure has not been covered.

The fish kidney is an integrated multi-functional organ that plays roles in osmoregulation and immunological functions through hematopoiesis; both its morphology and histology have been frequently described (Groman, 1982; Esfandiyari et al., 2022; Senarat et al., 2022; Oh et al., 2023; Guo et al., 2024). Morphologically, the fish kidney has been classified in different ways by different investigators. Kumar et al. (2016) separated the kidney of *Heteropneustes fossilis* into anterior and posterior regions while the kidney of other species has been separated into anterior, middle and posterior regions (Bahmani et al., 2005; McCampbell et al., 2014; Supriyati et al., 2019). Morovvati et al. (2012) described the kidney of *Barbus pectoralis* as having a head, body and tail. However, all kidney regions present nephrons, which are considered the functional units of the kidney (Kent and Carr, 2001) and have crucial roles in the filtration of blood and water (Feist, 2009). Nephrons are composed of the renal corpuscle and renal tubules with histologically distinguished proximal and distal convoluted tubules (Kumar et al., 2016; Esfandiyari et al., 2022; Senarat et al., 2022).

The main functions of the kidney are hematopoiesis and immune response activity (Oh et al., 2023). The anterior part of the fish kidney is analogous to the mammalian adrenal gland (Takashima and Hibiya, 1995; Fernandes et al., 2019). It is important to remember that studies of teleost head kidneys have highlighted their important role with respect to immune cells, differential hematopoietic activity and blood cell formation (Willett et al., 1999; Shahsavani and Mohri, 2004; Roberts and Ellis, 2012; Witeska et al., 2023). The importance of the fish kidney and its functions during ontogenesis and hematopoiesis have been confirmed histologically in *Clarias gariepinus*, *Sarotherodon mossambicus* (Boomker, 1979) and *Ictalurus punctatus* (Fijan, 2002a). Zapata et al. (1996) revealed mature granulocytes and agranulocytes in the hematopoietic tissue of fish under light microscopic (LM) observation. Later, Gangopadhyay and Homechaudhuri (2011) described erythropoietic and leucopoietic cell populations and their organelle activities in a TEM-based ultrastructural study of the head kidney of *Clarias batrachus*, and Kumar et al. (2016) identified erythrocytes, macrophages, neutrophils, granulocytes, monocytes and basophils in the head kidney of *H. fossilis*. However, the standard morphogenesis of the hematopoietic series of catfish in aquaculture is unclear and needs to be clarified.

Recent hematological investigations have shown that peripheral blood cells are generated from hematopoietic tissue (Chew and Gibson-Kueh, 2023; Witeska et al., 2023). An integrated overview of these features is necessary as a diagnostic tool to assess the physiological response and health of fish in both field and laboratory (Megarani et al., 2020; Siddiqui et al., 2020; Witeska et al., 2022). Witeska et al. (2022) confirmed that blood parameters are an easy and sensitive method of assessing changes in fish health status. More specifically, the morphology and morphometry of blood cells have been investigated in *Danio rerio*, *Cyprinus carpio carpio* (Megarani et al., 2020), *Ambassis nalu*, *A. vachelli*, and *Inegocia japonica* (Imsonpang et al., 2024). However, the determining descriptive

blood parameters for fish must first be established with a focus on routine blood morphology (Blaxhall and Daisley, 1973; Friedrichs et al., 2011).

In this study, we therefore evaluated the kidney structure, hematopoietic histology and ultrastructure between kidney regions of *C. nieuhofii* with the aim of investigating blood cell formation. This study focuses on the male fish, which are commonly kept in the hatching site, and which display faster growth than the female fish. Our findings could be a useful tool for studying kidney functions of *C. nieuhofii* in aquaculture.

MATERIALS AND METHODS

Six sexually mature male *C. nieuhofii* [55.0 ± 0.4 cm total length (mean $\pm$ SD)] without lesions or parasites were selected from fish reared at the breeding station, Faculty of Agricultural Technology, Songkhla Rajabhat University, Thailand. All fish were kept apart, stocked in 6 blue polyethylene tanks (Length $\times$ Width $\times$ Depth = $57 \times 38 \times 26$ cm). Each fish was kept in the standard conditions of temperature (28 ± 1 °C), dissolved oxygen (6.15 mg/L), pH (6.0 ± 0.8) and total ammonia nitrogen (< 0.1 mg/L; $\text{NO}_2\text{-N} < 0.1$ mg/L), which were monitored every day. Thirty percent of the water in the tanks was replaced with fresh water once every week. Twice daily at 08.00 h and 16.00 h, the fish were fed CP 9922 Catfish Feed 20 KG (CPF Co., Ltd., Type I-F1).

Sampled fish were euthanized by rapid cooling (Wilson et al., 2009). Using a $21\text{G} \times 1''$ needle following the standard method (Watson et al., 1989), blood was drawn from the isthmus through the pericardial cavity into heparin tubes. Cytochemical blood cell smears were stained with the dip quick staining method and the Alcian Blue/Periodic acid-Schiff (AB/PAS) method to detect structural and chemical components. Three thousand blood cells from blood cell smears were counted as the percentage proportion (blood cell type/total number $\times 100$). The cell sizes were observed morphometrically according to erythrocyte length (EL) and white blood cells, by measuring 20 randomly-selected cells following Knowles et al. (2006) and Wang et al. (2021). The morphometric data above were calculated as means $\pm$ SD. Blood cell cytochemistry was examined and photographed using a 3DHISTECH Panoramic Viewer (3DHISTECH, Hungary).

The sampled fish were dissected through the body longitudinally. Five samples of kidney tissue from each fish were resected at a size of 0.5×0.5 cm for further studies. All five tissue samples per fish were fixed in 10% neutral buffer formalin for 24 h at room temperature, dehydrated in ethanol and embedded in Paraplast (Leica, Germany), following the standard histological techniques (Presnell and Schreiber, 2013; Suvana et al., 2013). To observe the histological structures of kidney tissues, paraffin sections $4 \mu\text{m}$ thick were stained with Harris's hematoxylin and eosin (H&E) and picosirius red. To detect apoptotic cells, unstained sections were tested and processed for TUNEL assays. They were treated with a blocking solution containing 10% normal goat serum (NGS; Vector Laboratories) and 4% bovine serum albumin. TUNEL detection was carried out using the DeadEnd™ Colorimetric TUNEL System according to the manufacturer's instructions. Samples were incubated in TdT buffer at 37 °C for 1 h. The histological and TUNEL slides were examined and photographed as described above. The human liver sections are used as the positive controls.

A small remaining portion of head kidney tissues were pre-fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4 °C for 12 h overnight and then post-fixed in 1% osmium tetroxide (OsO_4 , 1%v/v) (Sigma-Aldrich, St. Louis, USA) for 2 h. These samples were rinsed, dehydrated with increasing concentrations of acetone and embedded in Araldite® (Sigma-Aldrich). All plastic blocks were sectioned at a thickness of 60 to 90 nm using an ultramicrotome (EM UC7, Leica, Inc., Wetzlar, Germany). These sections of unstained kidney were

double-stained with uranyl acetate and lead citrate for observation with electron microscopy (Philips/FEI Tecnai G2 F20, FEI Co., Eindhoven, Netherlands).

All procedures were performed under the supervision of the Songkhla Rajabhat University Animal Care and Use Committee under process number IACUC-F004/2568.

RESULTS

Kidney structure and hematopoietic series

The kidney of *C. nieuhofii* was elongated, forming a paired triangular mass running medially along the vertebrae (Figures 1A-1C). The parenchymal anterior, or head, kidney (AK) was composed only of hematopoietic tissue, whereas the posterior kidney region (PK) contained renal tubules with less interstitial hematopoietic tissue and fewer nephrons (Figures 1D-1F). Melanomacrophage centres (MMCs) with aggregations of highly pigmented phagocytes were found in both regions (Figures 1D and 1F). Notably, hematopoietic cells were mostly found in the AK, and were rarely labeled by the TUNEL assay (Figures 1G-1H), unlike other tissues (Figure 1I). A summarized diagram of the renal corpuscle and its tubule is presented in Figure 1J. The structure should be clarified in further observations.

LM observation revealed that the renal tubules of *C. nieuhofii* were divided into two sequential convoluted tubule segments: the proximal convoluted tubule (PT) and the distal convoluted tubule (DT) (Figure 1F). Both segments were confluent to the collecting duct (Data not shown). The PT was linked with the renal corpuscle and was lined with a simple cuboidal epithelium with a prominent brush border on the apical surface (Figure 1F). TEM revealed that the epithelial cells of the PT contained an oval nucleus positioned centrally (Figure 2A). A well-developed brush border was identified in the apical zone of epithelial cells (Figures 2B-2C), and the cytoplasm presented prominent basal membrane infoldings associated with mitochondria (Figures 2D-2E).

Under TEM, the hematopoietic series of the pronephros could be classified into erythropoiesis, granulopoiesis and lymphopoiesis. All the series were present together and clearly visible.

Erythropoiesis

Erythropoiesis, unlike the other hematopoietic series, was mainly observed in the AK. TEM imaging of erythroid cells (Figure 3A) provided evidence of erythropoiesis, granulopoiesis and lymphopoiesis. Mature elongated erythrocytes presented homogeneous electron-dense cytoplasm. The cytoplasm of the first morphologically recognizable proerythroblast (Figure 3B) presented a prominent oval nucleus, rough endoplasmic reticulum (RER), mitochondria, ribosomes and small vacuoles. Erythroblasts presented some RER and small vacuoles (Figure 3C). Young developing erythrocytes presented small vacuoles but were without organelles (Figures 3D-3E).

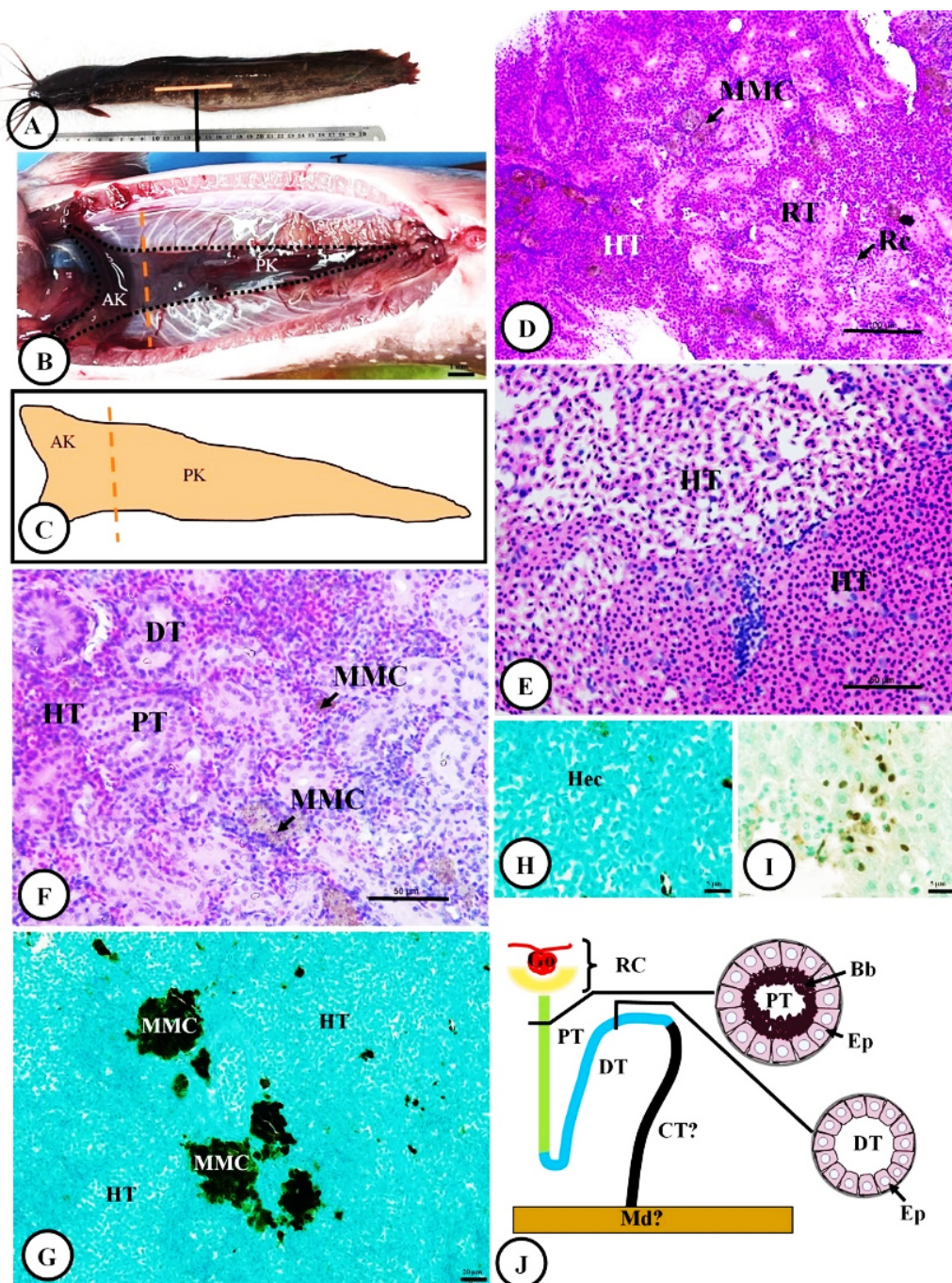


Figure 1 Light microscopic level images show the kidney structure and hematopoietic tissue of *C. nieuhofii*. A-C: The kidney had two regions: the anterior kidney (AK) and posterior kidney (PK). The black dot line in 1B delineates the kidney area, the orange dash line in 1C divides the AK from the PK. D-F: The renal capsule (Rc), hematopoietic tissue (HT) and melanomacrophage centres (MMC) in the posterior kidney. The proximal (PT) and distal (DT) convoluted tubules in the PK. G-H: Some of the rare TUNEL-positive hematopoietic cells in the AK. I: A more typical TUNEL-positive section of human liver. J: A summarized diagram of the renal corpuscle (RC) and the renal tubules. The collecting duct (CT?) and mesonephric duct (Md?) could be investigated in further studies.

Abbreviations: Bb = brush border, Ep = epithelium, Go = glomerulus, Hec = hematopoietic cell, RT = renal tubule

Staining methods: D-F = Harris's hematoxylin and eosin (H&E) and G-I = TUNEL assays.

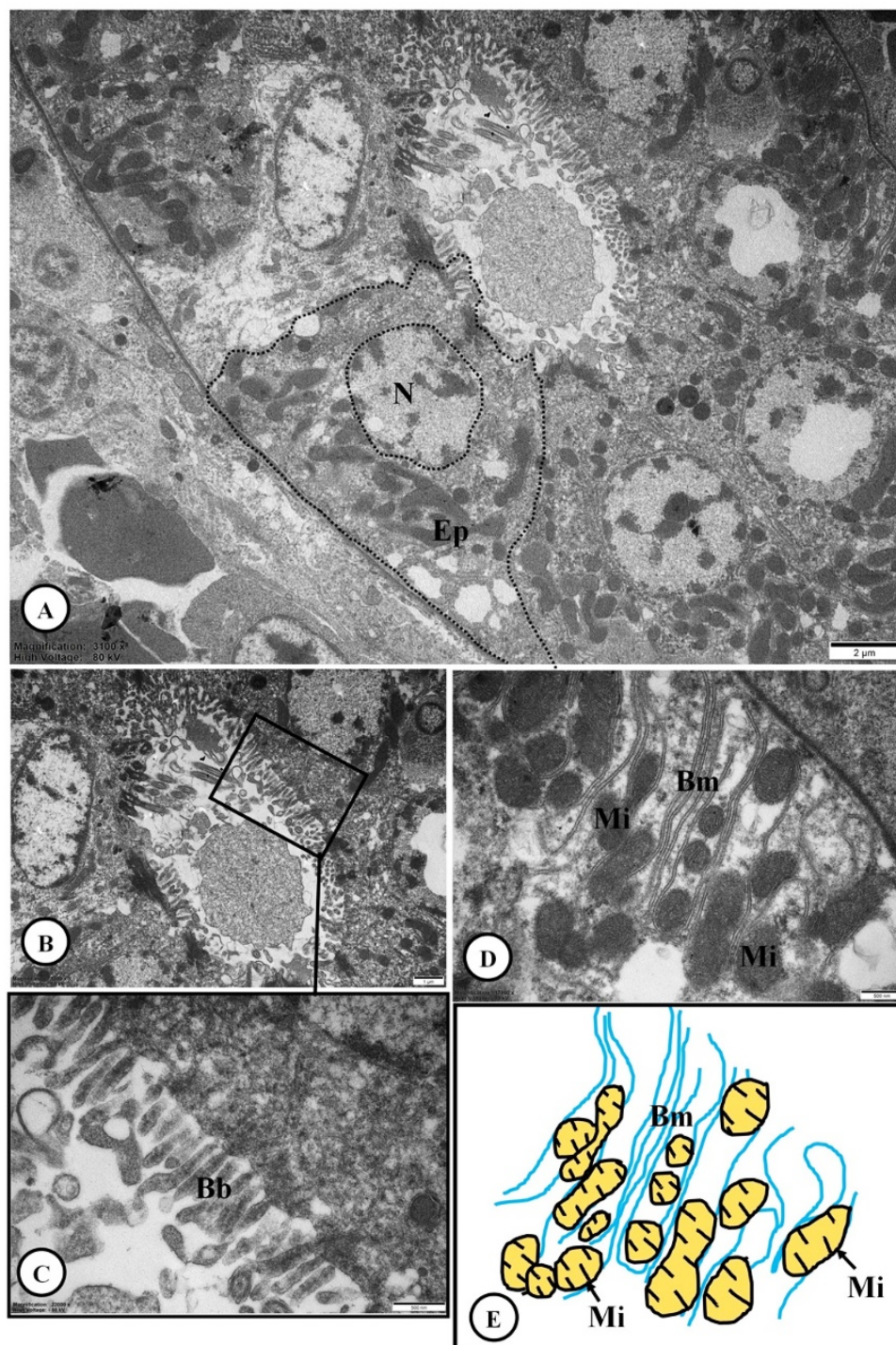


Figure 2 Ultrastructure of a representative renal tubule of the *C. neuhofii* kidney. A: A thin cross section shows the proximal convoluted tubule lined with simple cuboidal epithelium (Ep). B-C: Well developed brush border (Bb) in an epithelial cell. D-E: The cytoplasm contained prominent brush border basal membrane infoldings (Bm) associated with mitochondria (Mi). Abbreviation: N = nucleus.

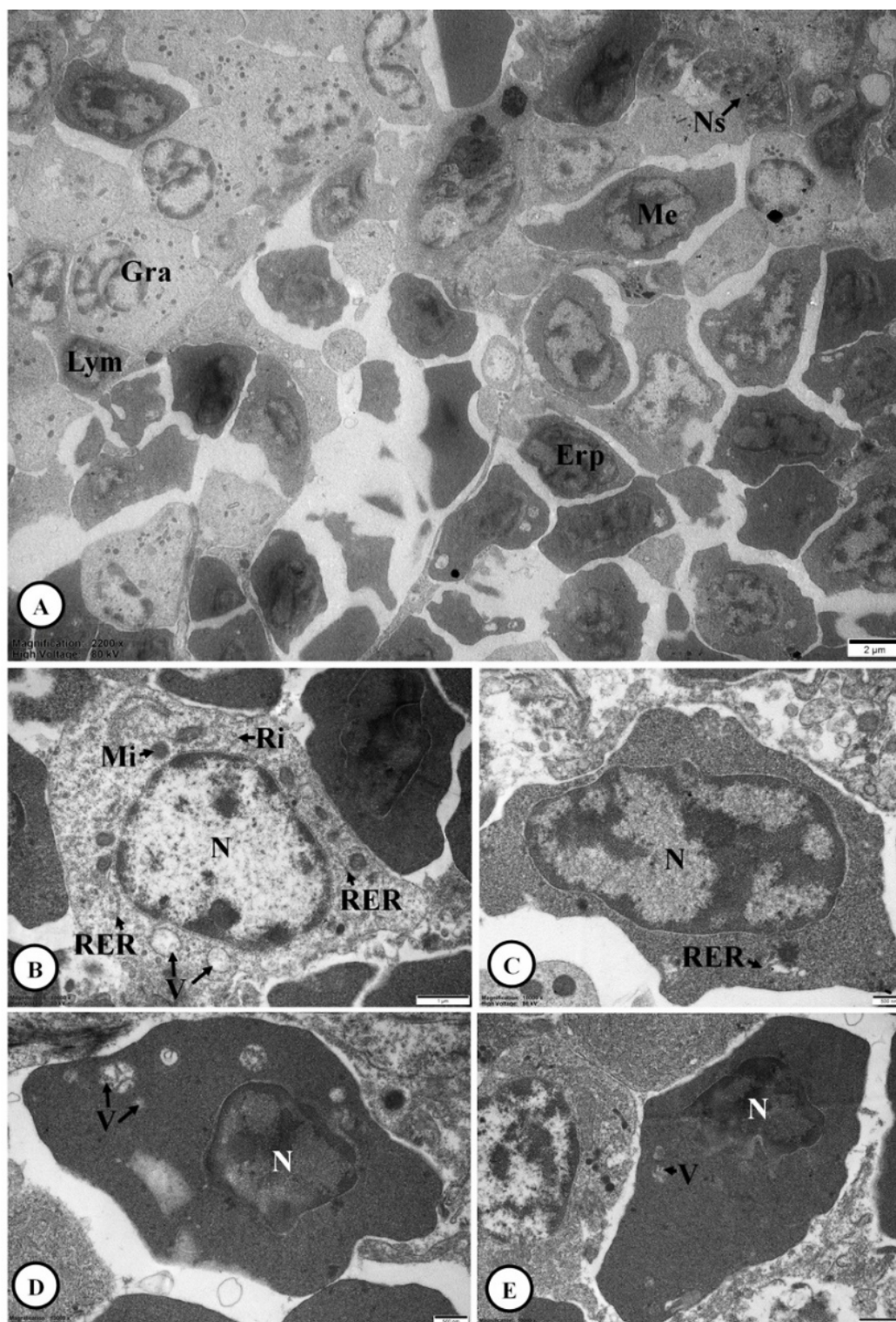


Figure 3 A: Erythropoiesis (Erp), granulopoiesis (Gra) and lymphopoiesis (Lym) were present simultaneously in the *C. nieuhofii* kidney. B: The formation of the proerythroblast. C: Erythroblast. D-E: Young erythrocytes. Abbreviations: Me = mature erythrocyte, Mi = mitochondria, N = nucleus, Ns = neutrophil-like structure, RER = rough endoplasmic reticulum, Ri = ribosome, V = vacuole.

Granulopoiesis

Based on cell shapes and cytoplasmic constitutions, the granulopoietic series could be divided into three cell types, including myeloblasts, promyelocytes and granulocytes. The series was more easily observed in the AK than the PK.

Myeloblasts had a characteristically prominent nucleus with prominent nucleoli (Figure 4A). The cytoplasm of this stage presented the formation of free ribosomes, prominent mitochondria and a few strands of RER (Figure 4A). Promyelocytes were characterized by the presence of highly condensed heterochromatin in the nucleus while the cytoplasm was mainly made up of the important organelles including RER, mitochondria and ribosomes (Figure 4B). Granulocytes were the final stage of granulopoiesis (Figure 4C) but specific granules and nucleic shapes were observed in several views (Figures 4D-4F). Our observations revealed a neutrophil-like structure (NS) (Figures 4D-4E) and an eosinophil-like structure (ES) (Figure 4F). The NS contained a multi-lobed nucleus, ribosome, RER and mitochondria. Granules of different sizes were identified. The ES had a kidney-shaped nucleus, and the cytoplasm contained different types of granules, with electron-dense oval and crystalline features, and mitochondria.

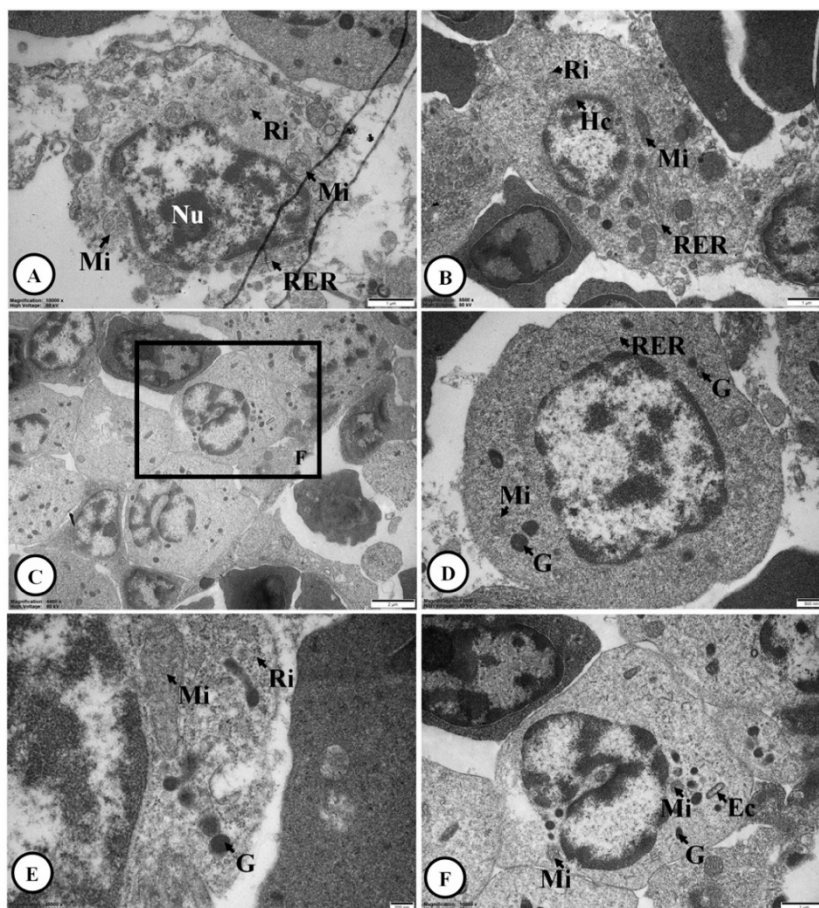


Figure 4 Ultrastructure of the granulopoiesis of the *C. nieuhofii* kidney. A: Myeloblast. B-C: Promyelocyte. D-E: Granulocytes with a neutrophil-like structure showing prominent electron-dense granules (G). F: A granulocyte with an eosinophil-like structure with electron-dense granules (G) and electron-dense crystalline features (Ec).

Abbreviations: Hc = heterochromatin, Mi = mitochondria, Nu = nucleolus, Ri = ribosome, RER = rough endoplasmic reticulum.

Lymphopoietic series

The lymphopoietic series could be divided into two stages: lymphoblasts (Figure 5A), and lymphocytes (Figures 5B-5C). The lymphoblasts were large round cells with an oval nucleus containing granular condensed heterochromatin (Figure 5A) and organelles including free ribosomes, RER and a few large mitochondria (Figure 5A). The lymphocytes could be classified into large and small types. The large type (Figure 5B) had an irregular shape. The nucleus contained a large block of heterochromatin, and the cytoplasm contained numerous RER and mitochondria. The small type (Figure 5C) had an oval nucleus and prominent heterochromatin blocks.

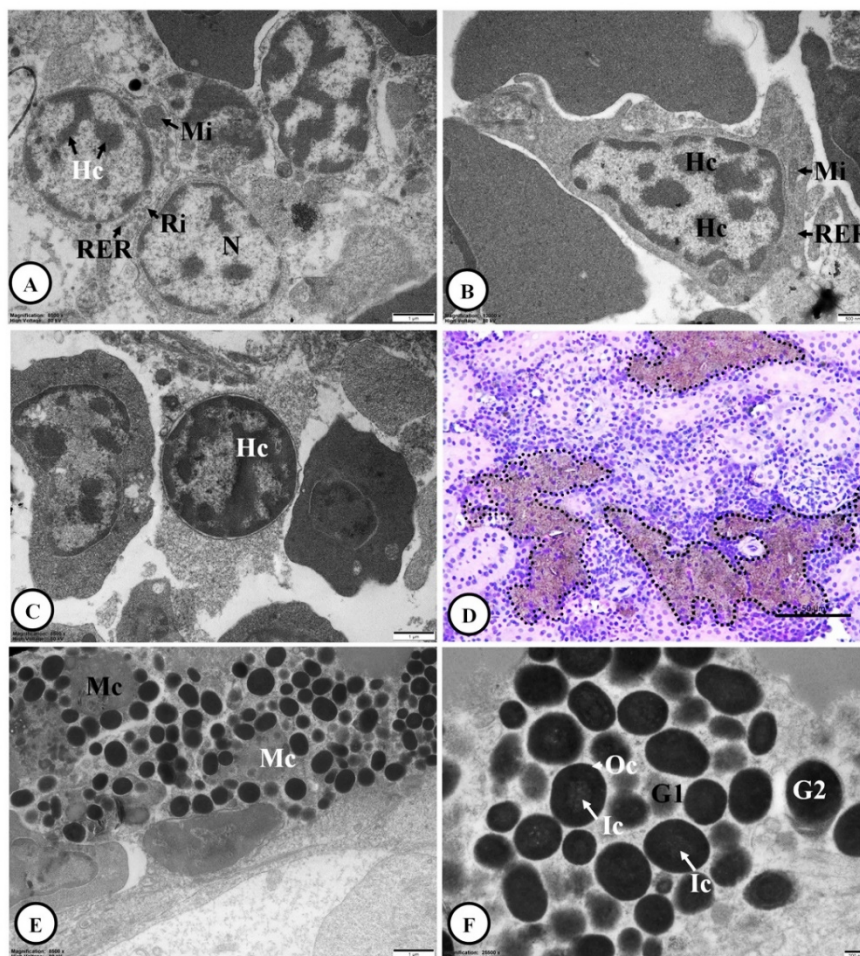


Figure 5 Structure and ultrastructure of the lymphoplasmopoiesis and melanomacrophage centres of the *C. nieuhofii* kidney. A: Lymphoblasts. B: Large lymphocytes. C: Small lymphocytes. D: A melanomacrophage centre (MMC, black dash line) in the posterior kidney. E: Granules of different sizes and macrophages (Mc). F: Type I granules (G1) and type II granules (G2). The G1 is identified by its less electron-opaque characteristic and the G2 by the highly electron-dense granule in the outer zone (Oc) and less electron-dense granular cortex in the inner zone (Ic).

Abbreviations: Hc = heterochromatin, Mi = mitochondria, N = nucleus, RER = rough endoplasmic reticulum, Ri = ribosome.

Staining methods: D = Harris's hematoxylin and eosin (H&E).

Melanomacrophage centres

MMCs were easily identified among the hematopoietic tissue in both kidney regions, but it was more prominent in the AK (Figures 5D). Structures of aggregated macrophages with various accumulated pigments (Figures 5E) presented several engulfed structures together with granules of different sizes and types (Figure 5F). The granules could be classified into two types: G1 and G2 (Figure 5F). G1 granules were visible among the G2 granules and were less electron-opaque (Figure 5G), whereas G2 granules were highly electron-dense in the outer zone (a) and less electron-dense in the thinner granular cortex (Figure 5F).

Morphological characterization of peripheral blood cells

High-magnification LM observation and the percentage of each blood type revealed that erythrocytes of *C. nieuhofii* were mostly oval (95.3 ± 3.64 % of total blood cells) unlike the other blood cells. The oval nucleus-stained magenta, and the cytoplasm pink (Figures 6A-6C). Morphometrically, the EL of this blood cell was 11.48 ± 0.69 μm (Table 1). Four types of leucocytes were identified in blood cell smears, including heterophils, neutrophils, small and large lymphocytes, and monocytes (Figures 6D). Heterophils made up the highest proportion of leucocytes (1.56 ± 1.63 % of total blood cells and 32.21 % of total white blood cells). They were oval with an eccentric nucleus (Figure 6D) and stained pink (a positive reaction to AB-PAS staining) (Figure 6E). Neutrophils had an EL of 12.11 ± 0.60 μm , and were round with a bilobed, or trilobed nucleus (Figures 6F-6G and Table 1).

Small granules presented a pink cytoplasm, indicating a positive reaction to AB-PAS staining (Figures 6H-6I). Lymphocytes were widely distributed (1.16 ± 1.13 % of total blood cells and 24.21 % of total white blood cells). Monocytes had an EL of 13.06 ± 1.35 μm . They made up the lowest proportion of leucocytes (0.9 ± 0.75 % of total blood cells and 18.95 % of total white blood cells). They were oval, having a large, eccentric, reniform nucleus (Figures 7A-7B) filled with basophilic nucleoplasm (Figure 7A). In this study, the lymphocytes could be divided into large and small lymphocytes (Figures 6I and 7C-7D). Both types had a large centrally located nucleus and were characterized by the presence of small amounts of cytoplasm (Figures 6I and 7C-7D); however, their cell sizes differed (8.6 ± 0.60 μm for large lymphocytes and 6.83 ± 0.60 μm for small lymphocytes; Table 1). Thrombocytes were still visible in both isolated cells and groups of cells (Figures 7E-7F). Each cell was oval and had a large nucleus (Figure 7F).

Table 1 The table compares the size of peripheral blood cells of *Clarias nieuhofii* (Mean $\pm$ SD, μm , N = 30 per the pooled erythrocytes and N = 20 per pooled leucocytes) and other catfishes

Blood cell morphometry	Present study (μm)	<i>Ictalurus punctatus</i> (Fijan, 2002b)	<i>Pelteobagrus fulvidraco</i> (Chen et al., 2019)	<i>Heteropneustes fossilis</i> (Pattanaik et al., 2022)
Erythrocyte length	11.48 ± 0.69	11.0 ± 1.0	12.22 ± 0.92	10.94 ± 1.5
Heterophils	12.98 ± 0.67	-	-	-
Neutrophils	12.11 ± 0.06	-	10.97 ± 1.43	-
Small lymphocytes	13.06 ± 1.35	-	8.79 ± 1.52	-
Large lymphocytes	6.83 ± 0.58	-	6.37 ± 0.78	-
Monocytes	9.68 ± 1.10	-	9.80 ± 0.81	-

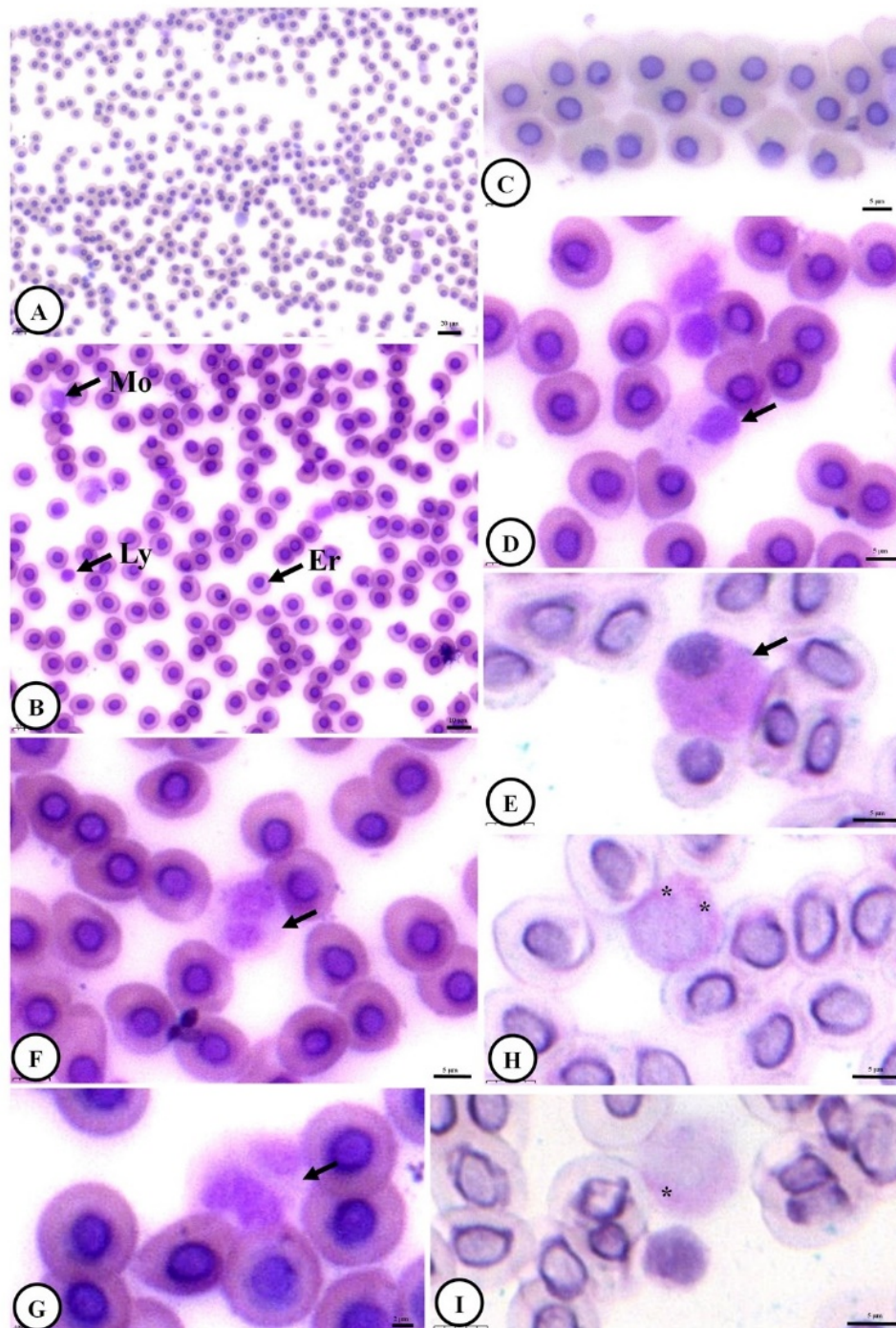


Figure 6 Microstructure of peripheral blood cells in *C. nieuhofii*. (A-B) Overview of blood cell smear with representative blood cells: erythrocyte (Er), lymphocyte (Ly) and monocyte (Mo). High-magnification images of (C) erythrocytes, (D-E) heterophils, and (F-G) neutrophils show positive reactions (asterisks) to staining near large lymphocytes. Staining methods: A-D, F-G = dip quick staining and E, H, I = Alcian Blue/Periodic acid-Schiff (AB/PAS).

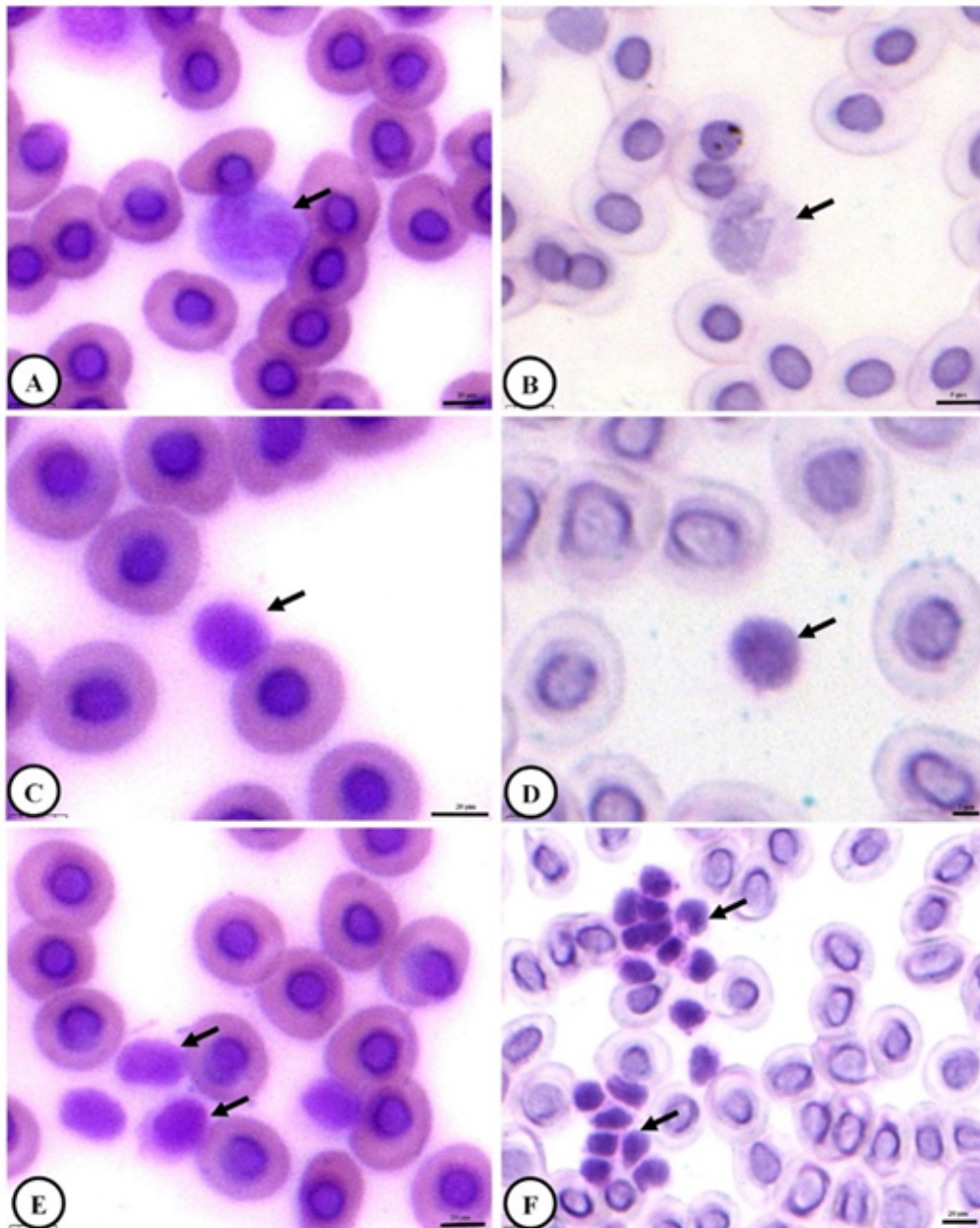


Figure 7 Microstructure of peripheral blood cells in the kidney of *C. nieuhofii*. High magnification images show monocytes (A-B), small lymphocytes (C-D) and thrombocytes (E-F). Staining methods: A, C, E = dip quick staining and B, D, F = Alcian Blue/Periodic acid-Schiff (AB/PAS).

DISCUSSION

Farmed fish are susceptible to disease and physiological imbalance due to the stressors that are common in aquaculture (Schreck et al., 1995; Mesa et al., 1998). Contributing factors cited in the literature include rearing density and environmental changes (Clarke et al., 2013). However, since the sampled *C. nieuhofii* were kept individually and reared in the standard environmental condition for aquaculture, these factors could not have influenced the observed kidney hematopoiesis and blood cell proportions.

The anatomical and histological investigations of the kidney of several fish species (Bahmani et al., 2005; McCampbell et al., 2014; Supriyati et al., 2019), including *Salmo trutta fario*, *C. Idella* (Morovvati et al., 2012) and *B. sharpyie* (Moradi, 2005), have highlighted the presence of apoptotic cells in the kidney as well as the importance of morpho-functional studies. We first used the TUNEL assay to detect the presence of apoptotic hematopoietic cells in *C. nieuhofii*, but positive results were rare. This finding suggested that the hematopoietic cells of *C. nieuhofii* were healthy as a result of programmed cell death (PCD), and represented a profoundly important homeostatic mechanism (Kerr et al., 1972; Wyllie, 1987). The rare TUNEL-positive hematopoietic cells of *C. nieuhofii* might be a response to environmental change (reviewed by AnvariFar et al., 2017), food contaminants or toxicants (Dallinger et al., 1987); however, it is important to note that the above factors need to be studied in further observations.

The hematopoietic tissue of teleosts is most commonly divided into erythropoietic, myelopoietic, lymphopoietic, and thrombopoietic lineages (Belosevic et al., 2006; Abdel-Aziz et al., 2010; Witeska, 2013); however, the monocytopenia and thrombopoiesis of *C. nieuhofii* are relatively unexplored still. It is possible that the different categorizations of hematopoietic series might be related to individual preferences of criteria, as mentioned in the introduction to this work, or rare/unclear features found in TEM studies. We hope that the present data of *C. nieuhofii* represents the first observation of these processes in the genus *Clarias*.

Our observations of the erythropoiesis of *C. nieuhofii* identified a series from proerythroblasts to young erythrocytes that enabled the confirmation of typical cytological changes such as increased heterochromatinisation and a reduction of cytoplasmic organelles. These features were less evident than previously reported for other fish species (Witeska, 2013; Zhong and Yan, 2025). Among the organelles in the erythropoietic stage of *C. nieuhofii*, we observed that the accumulation of ribosomes increased from the proerythroblast to young erythrocyte stages, indicating the synthesis of hemoglobin (Majeti et al., 2007). It should be noted that the ribosome is the marker of erythrocyte maturity (Tooze and Davies, 1976).

Several studies have reported the granulopoietic series of different species (Yamamoto and Iuchi, 1976; Mattisson and Fänge, 1977; Flerova and Balabanova, 2013). Our observations of granulopoiesis in *C. nieuhofii* indicated several distinctive aspects of granule characteristics and nucleic morphology (Flerova and Balabanova, 2013) that have been described previously (Savage, 1983; Zuasti and Ferrer, 1988; Meseguer et al., 1990; Senarat et al., 2020). These differences may be attributed to the regulatory mechanisms and physiological roles of the trans-Golgi network in the immune function (Borregaard and Miller, 1987; Lawrence et al., 2018). Additionally, the final product of granulopoiesis included heterophilic (neutrophilic) and eosinophilic structures, which were noted in previous reports of the ultrastructure (Zuasti and Ferrer, 1988; Meseguer et al., 1990). The key characteristic of these cells was the appearance of homogeneous electron-dense granules; however, some investigations of fish granulocytes have found two types of granules including the granular and fibrillar (Zuasti and Ferrer, 1988). We found that the kidney-shaped nucleus of the eosinophil-like granulocyte structures also contained two types of granules—characterized by electron-dense oval features and crystalline features. It is now recognized that the findings of Zuasti and Ferrer (1988) are unclear since the eosinophil-specific granules of these structures contained a peroxidase, eosinophil-derived neurotoxin and cationic protein that might be supported by the physiological activity of the fish.

The present study did not find evidence of the thrombopoiesis described in other species of fish (Barber and Westerman, 1981; Zuasti and Ferrer, 1988). Esteban et al. (1989) reported the exclusive thrombopoiesis (immature prothrombocytes, mature prothrombocytes and thrombocytes) in the AK of *M. saxatilis*. It has been suggested that the main production of thrombopoiesis in the AK contributes to the efficient hemostatic mechanism of blood coagulation in fishes

as fibrinogen is converted to fibrin (Ortiz and Esteban, 2024). The occurrence of thrombopoiesis in *C. nieuhofii* needs to be investigated in further study.

MMCs have been widely observed in the kidneys of various fish species (Tsuji and Seno, 1990; Gandahi et al., 2020; Senarat et al., 2022). In the present work, the histological study and TEM clearly identified MMCs among hematopoietic tissue and renal tubules, as reported in *O. niloticus* (Abdel-Aziz et al., 2010) and *D. rerio* (Gandahi et al., 2020). According to previous observations, the presence of MMCs may be associated with erythrocyte-degraded hemoglobin (Agius and Agbede, 1984; Zuasti and Ferrer, 1989), foreign particle-solidification (Nakamura et al., 1993), and free radical reactions (Agius, 1985). Notably, it is possible that the kidney tissue of *C. nieuhofii* may also contain foreign antigens, making it possible to identify biotic and abiotic parameters. However, variations may occur depending on the species, age, reproductive period, and environmental conditions (Agius, 1985; Steinell and Bolnick, 2017).

LM level observation revealed nucleated erythrocytes, as reported generally in fish species (Fang et al., 2014; Yilmaz et al., 2015), in contrast to mammalian erythrocytes, which lack a membrane-bound nucleus (Ji et al., 2008). The size and abundance of erythrocytes are correlated with hemoglobin levels, which are strongly influenced by environmental conditions that change oxygen demands (Maciak and Kostecka-Myrcha, 2011). The association between erythrocyte size and variation in metabolic activity was addressed by Smith (1925). Increased cell size has since been associated with adaptive reductions and oxygen transport (Witeska, 2013; Dal'Bó et al., 2015; Val, 2000). The examination of erythrocyte size of the sampled catfish strongly supported the findings of Venkatachalam et al. (2012) in a study of *C. batrachus*. The length of *C. nieuhofii* erythrocytes in the present work was greater than that reported for *Ictalurus punctatus* (Fijan, 2002b) and *H. fossilis* (Pattnaik et al., 2022), but smaller than that of *Pelteobagrus fulvidraco* (Chen et al., 2019) (Table 1). We therefore suggest that the large erythrocytes observed in *C. nieuhofii* must be an adaptation to increase oxygen uptake and transport efficiency. Importantly, we provided the hematological profiles, and erythrocyte size will be recognized as a valuable tool for the future monitoring of fish health.

We observed four types of white blood cells in the blood smears of *C. nieuhofii* but the most abundant were the heterophils for granulocytes and lymphocytes for agranulocytes. The terms heterophil and neutrophil in fish are used to describe granulocytes (Bianchi et al., 2014), but neutrophils are sometimes referred to as heterophils, including in a study of *C. carpio* and *C. auratus* (Groff and Zinkl, 1999). However, in fish, the distinction between heterophils and neutrophils, as well as the terminology applied to these cells, remains unclear and lacks standardization. In the present study, heterophils were considered to present an eccentric nucleus, and neutrophils a multi-lobed nucleus, as commonly found in some other fishes (Barber and Westermann, 1978; Tavares-Dias and Barcellos, 2005). It is noted that the high numbers of heterophils observed in *C. nieuhofii* implied that these fish might harbour infiltrated antigens since the functional structure of heterophils concerns the recognition, capture and destruction of pathogens (Genovese et al., 2006). Harvey (2012) suggested that the presence of heterophils was associated with a response to infectious or non-infectious inflammatory conditions. The health assessment of *C. nieuhofii* in captive conditions needs to be further developed.

The presence of relatively high numbers of lymphocytes in *C. nieuhofii* does not indicate that lymphocytes are the predominant leucocyte in fish. Lymphocytes of zebrafish made up 71–92 % of leukocytes (Murtha et al., 2003) while the most abundant leukocyte (49.13 %) of *Paralichthys olivaceus* could not be identified with certainty (Gao et al., 2024). High lymphocyte counts have been attributed to infectious diseases or impaired immunity under chronic exposure. The most abundant lymphocytes in this study were thought to be secreted in response to ecological health problems, and this finding is supported by Murtha et al. (2003).

The blood cell morphology of *C. nieuhofii* is presented in drawings and the cytochemical patterns of AB-PAS staining. The positive reaction to PAS found in the granules of neutrophils was associated with the storage of glycogen. Although found in four other fish species (Palić et al., 2011), this is the first unique pattern of cytochemical reactivity observed in *C. nieuhofii*. It is possible that glycogen is a vital source of cellular energy storage and defense against infection (Ueda et al., 2001).

CONCLUSIONS

The structural and histological features of the kidney and hematopoietic tissue of *C. nieuhofii* were characterized, and the hematopoietic process was clarified. Although, evidence of the monocytopenia and thrombopenia of the sampled catfish was limited, monocytes and lymphocytes were visible in the peripheral blood cells. Our findings provide useful descriptive health parameters for assessing renal function in aquaculture. Nevertheless, the practical application of these findings depends on further research to clarify the correlation between blood populations isolated via fluorescence/flow cytometry and the health monitoring of *C. nieuhofii* reared in sustainable catfish aquaculture practices in Thailand.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Saowalak Malawa: Writing – original draft (Lead), Methodology (Equal), Conceptualization (Equal), Writing – review & editing (Equal), Project administration (Lead).

Wijitra Tungse: Methodology (Equal) and Writing – review & editing (Supporting).

Natthaphon Rachuphimon: Methodology (Supporting) and Writing – review & editing (Supporting). Chanyut Sudtongkong: Formal analysis (Supporting) and Writing – review & editing (Supporting).

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Piyakorn Boonyoung: Formal analysis (Supporting), Investigation (Supporting) and Writing – original draft.

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