



Histological Description of the Electroreceptors and Other Key Tissues in the Black Ghost Knifefish, *Apteronotus albifrons*

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ABSTRACT

Black ghost knifefish is a unique model for studying many biological aspects such as development of electroreceptors, which help in determining the evolutionary history among species on the one hand, and on the other hand explains the mechanisms used by these species to communicate with each other. In this study, we described tissues of some organs in Black ghost knifefish *Apteronotus albifrons* that included the gills, liver, kidney, oesophagus, vertebrae, spinal cord and electroreceptors. The study characterized the electroreceptors that embedded in the skin in this species for the first time. The histological examination using Haematoxylin and Eosin stains reveals that this type of electroreceptors is tuberos.

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INTRODUCTION

In South America, there are many diverged species of fresh water fish, the estimated species have exceeded 5,000 000 [1, 2]. One of the most fascinating groups are the knifefish or Gymnotiformes which are characterised by their ability to produce electric fields and comprise nearly 200 species [3].

Gymnotiformes comprise three families, Gymnotidae, Apteronotidae and

Rhamphichthyidae, they live in tropical rivers distributed from Argentina to Mexico, and several rivers west of the Andes, and they live in abundance and diversity in the rivers of the Amazon and the Río de la Plata [4, 5]. They are inhabitant of a variety of aquatic environments, from large river channels, small streams, flooded forests, lakes and waterfalls [6, 7].

The Black ghost knifefish, *A. albifrons*, is a tropical fish belonging to the family Apterontidae, living and breeding in freshwater in South America, it spread in Venezuela, the Paraguay-Paraná River and the Amazon Basin [8]. Its body shape is relatively flat and laterally compressed and its back is smooth and finless. The predominant

colour of *A. albifrons* is black except the caudal fin, which is stick-like and has white rings, there is a white stripe on the back that starts from the head and extends to about the middle of the body, sometimes expanding into a stripe down its back [2] (Fig1).

The Black ghost knifefish, like other knifefish species, has remarkable sensory abilities. It also has a distinctive motion that allows it to swim forward, backward, sideways and vertically upward. It swims freely and at high speed in the water, among aquatic plants and rock crevices. Propulsion forces are generated by the undulations of the ventral fin that extend over most of the length of the ventral body surface. Maximum total length of the fish reaches 50 cm [9, 10].



Fig. 1. Black ghost knifefish, *A. albifrons*

Sensory systems can evolve in response to natural selection resulting from the effects of a variety of biotic and abiotic factors, species are affected by the environment in which they live and as a result some species developed lineage-specific sensory specializations, for example, some benthopelagic cavefish has poor eye that are less developed, but this is compensated for by taste buds, electroreceptors, and neuroblasts [11].

A. albifrons is considered a weak electric fish, with a long, flat trunk containing an electric organ that generates a continuous electrical discharge of the wave type Electric Organ Discharge (EOD). The discharges from the electric organ are limited to frequencies between 800 and 1200 Hz (about 1 kHz) and low amplitude (about 1 mV) [12]. The electric organ (EO), enable fish to produce their own electric field using specialized electric organs, these weak electrical charges are

used for communication and navigation purposes [13,14]. Issa et al. [15] studied the phenotypic and molecular characteristics of the electric ghost knifefish, and to know the effects of dexamethasone as a medical treatment on the regeneration of the severed tail. They concluded that it reduces the healing process in the injured tissues through its effect on signalling pathways for some genes responsible for regeneration process. In this study, we provide for the first time the histological description of some important tissues in the black ghost knifefish *A. albifrons* including the electroreceptors

MATERIALS AND METHODS

Animal ethical approval and Fish breeding

This study was conducted according to instructions and regulations of animal ethics of College of Agriculture / University of Basra. Black ghost knifefish (immature) were obtained from a local aquarium supplier in Baghdad / Iraq and transferred to a glass aquarium 60 litres under lab conditions: temperature 26°C, equipped with an air pump. They were fed twice daily, morning and evening, with dried blood worms and fish eggs (roe).

Histological approach

Knifefish were sacrificed by terminal anaesthesia with clove (500 mg.l⁻¹). Total length (mm) was recorded. Then, samples were fixed in 10% formalin 24 hours. After that, samples were washed with distilled

water to eliminate the fixative, then dehydrated with ascending concentrations of ethyl alcohol (70%, 80%, 90%, 100%) one hour each. After that, the samples were cleared with alcohol- xylene 1 hour and then xylene only 1 hour, then incubated in xylene-paraffin 60° C for 1 hour. Then, the samples were performed with paraffin wax 60° C overnight, and finally embedded with wax filled molds. Tissues were sectioned at 4 micrometres thickness using rotary microtome (Histo-line laboratories, Italy), and then the section strips were moved to a water bath (40°- 45° C) to make them to expand then loaded onto clean glass slides and stained with Haematoxylin and Eosin, and mounted with DPX. Slides photographed by a light microscope (Olympus CX 41, Japan).

RESULTS

As a part of sections near the skin in trunk region, show the tuberous electroreceptors embedded in the skin of black ghost knifefish that consist of sensory cells, which appear above the mass of fibre muscles (Fig.2).

The structure in fig. 3 accentuates the spinal cord of black ghost knifefish, spinal cord including grey matter with a number of electromotoneurons in the center of the spinal cord.

Fig. 4 shows the histological structure of the gills of the black ghost knifefish where they include the gill Lamellae, gill filaments, and the cartilage inside, with the presence of muscles at the beginning of the gill arch.

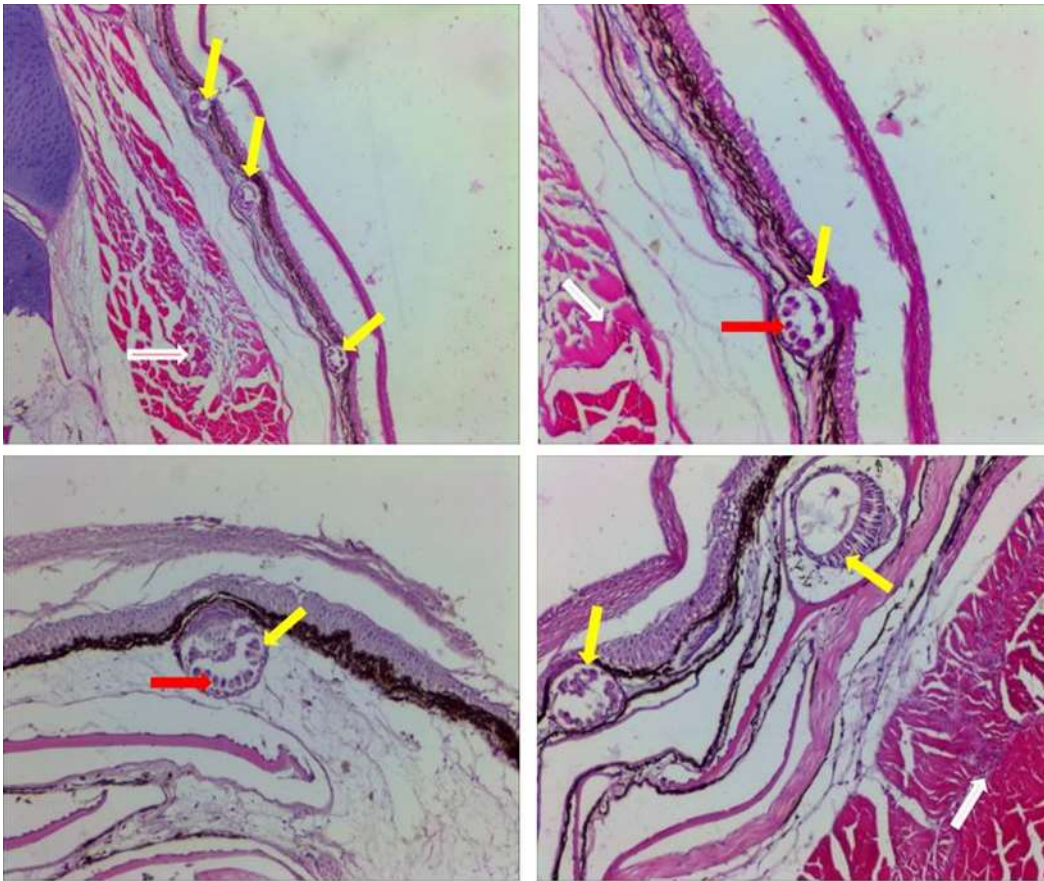


Fig.2. Cross section of black ghost knifefish near the skin, tuberosus electroreceptors (yellow arrow), sensory cells (red arrow), muscle fibres (white arrow). Hematoxylin & Eosin staining. 10x

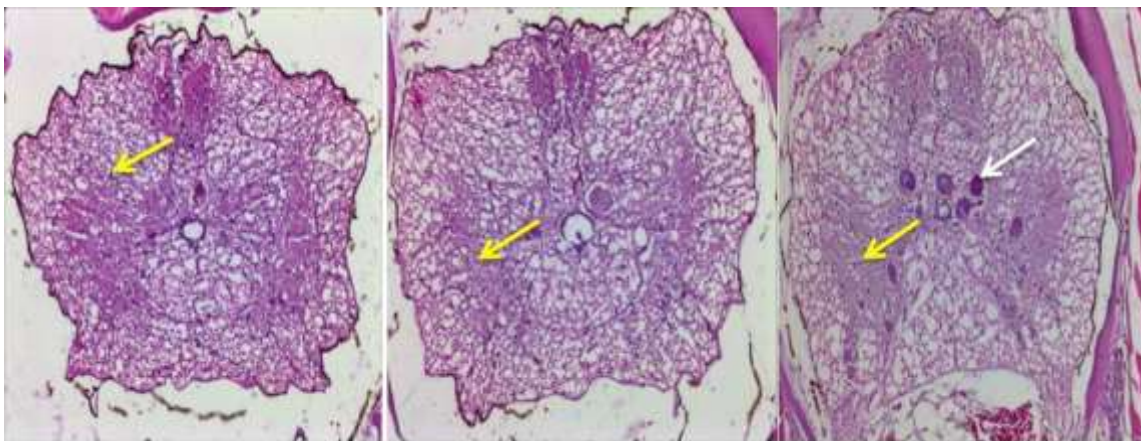


Fig. 3. Cross section through the spinal cord of black ghost knifefish, the gray matter (yellow arrow), electromotoneurons, (white arrow). Hematoxylin & Eosin staining. 10x

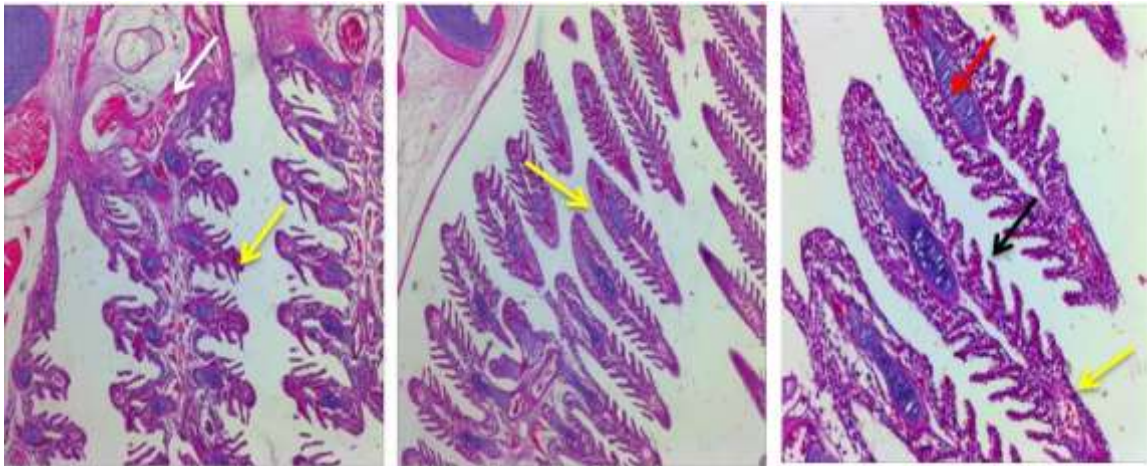


Fig .4. Gill tissue in the black ghost knifefish. Cartilage (red arrow), gill lamellae (yellow arrow), gill filaments (black arrow), muscle fibres (white arrow). Haematoxylin & Eosin staining. 10x

In the liver tissue section of black ghost knifefish, it is very clear to observe the squamous epithelial cells containing blood vessels and fibroblasts, they are divided into hepatic lobules of irregular size, with the apparition of vacuolar structures in

hepatocytes. In the histological structure of the kidney a group of glomeruli are clearly observed, within the mass of blood vessels that takes an oval shape, divided from each other. As for oesophagus, it is consisted of walls of layers from muscle fibres (Fig.5)

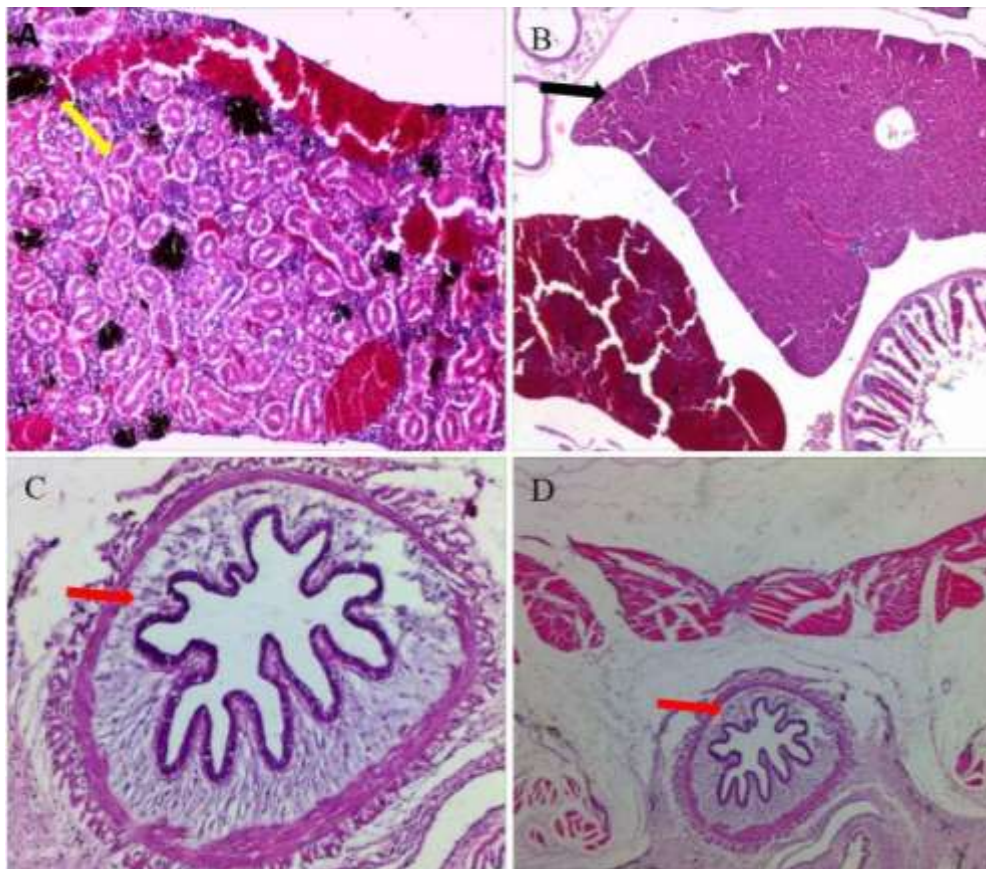


Fig. 5. Cross section of black ghost knifefish. A: Kidney, B: Liver, C&D: Esophagus. Hematoxylin & Eosin staining. 10x

The cross section in the vertebral displays the vertebra body and neural arches surrounding

and muscle fibres (Fig.6).

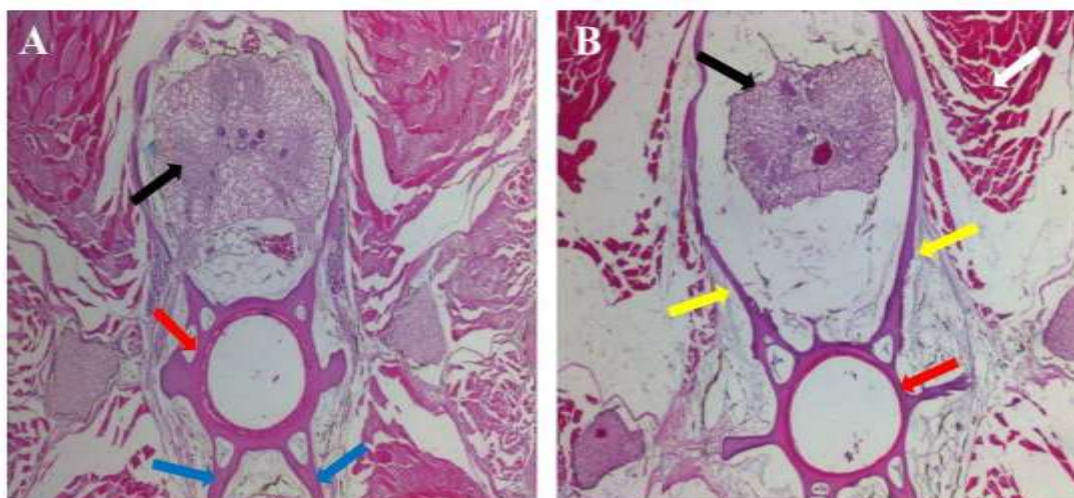


Fig. 6. Cross section (A&B) shows the vertebrae body (red arrow), neural arches (yellow arrow), hemal arches (blue arrow), spinal cord (black arrow), and muscle fibres (white arrow). Hematoxylin & Eosin staining. 10x.

DISCUSSION

Electroreceptors were described in ghost knifefish; they are like hole embedded in skin that contains sensory cells. Their function is receiving the electric signals from their family members or even other species that release the electrical charge. Through the histological description, it has been found that the type of electroreceptors is tuberous. These electroreceptors are distributed in many numbers on the body and more presence in head. Issa et al. [16] showed that the body of the electric ghost fish contains approximately 200 electroreceptors within the space of 1 cm diameters, electric ghost knifefish can detect the electric field through their receptors that are cluster of sensory cells in the skin, the tuberous electroreceptors are denser along the dorsal side nearly 2-3 times than those on the ventral surface and they are more sensitive [17]. The study of histology in *A. albifrons* is considered the first of its species, specially the determination of the type of

electroreceptors which varies between the members of its family. It was described that the electroreceptors in Gymnotiformes, Silurid, and Mormyrid, finding there are two types of electroreceptors, ampullary and tuberous [18]. In current study, the electroreceptor of *A. albifrons* was tuberous type. This type of receptor briefly consists of large receptor cell located in a well near the skin and covered with microvilli, its structure looks a like flap cup. This structure grants the receptor to receive and sense the charges from their sisters for communication to each other. This study has also conducted a description of several tissues and explained the structure of some organs. In general, gill of Osteichthyes constructed of arciform structure called arches, it supports the primary lamella that is two rows of twin filament, a holobranch is paired hemibranchs in gill of each side; There are four holobranchs in each gill at the sides of fish head [19].

The lamellae support and spread on the inner front edge of the gill arch forming

large number of small structures called teeth whose function is preventing food particles from passing to the gills, in addition extracting food and filtering it from the water, as well as the process of gas exchange [19]. Gill arches of the electric black ghost knifefish are slightly curved, and as in the boniest fishes consists of a cartilaginous structure surrounded by a mucous membrane supplied by blood vessels and muscle fibres [20] and [21]. And due to its carnivores feeding behaviour their gill teeth spaced and sturdy. As distinctive feature in such electric fish, the spinal cord includes structures called electroreceptors and sensory cells, that surrounding vertebrae, and the grey matter in the spinal cord [22] examined proliferation of cells in the spinal cord after transection process in the *Apteronotus leptorhynchus*.

Eiras-Stofella et al. [23] stated the presence of a large number of electromotoneurons in the spinal cord in *Apteronotus leptorhynchus* 162 days old, 52 mm long. There are several of fish species were selected as a model to study of nervous system characteristics, for example the lampreys that has long been studied for two features, in neuroscience, they possess large neurons including several classes of giant “specific” neurons located in the brain, whose large axons project into the spinal cord, these giant neurons and their axonal fibres contribute to understand electrophysiological aspects in organisms and open the horizons for applied sciences; lampreys have been considered among the most fundamental vertebrates for the study of central nervous system evolution [24].

The liver in black ghost knifefish showed parenchymal lobular regulation, externally surrounded by connective tissue. [25, 26] showed that liver tissue consists of connective tissue and is divided into irregular lobes. As a part of digestive system,

oesophagus is the first part of digestive system, it consists of epithelium cells and tunica mucosa. In fish, the muscular structure of oesophagus allow the passage of food, digestion, and regulation of osmotic pressure [19], therefore, the mucous cells observed in the studied fish may act as a mechanical and chemical barrier against microorganisms as bacteria and viruses [19, 27].

Genten [28] showed that mucous cells that are in the oesophagus are found in high abundance in neotropical fishes. Kidney appears as circular structures of a strip with a red color. Kidney in fish consist of many units called capsules which represent the nephrons, they are made of connective tissue and they are red in color, and contain small peripheral structures named glomeruli [29]. [30] indicated that the percentage of blood tissue in the kidneys is consistent with the distribution of urinary tubules and glomerulus. Hoar and Randall [31] described the kidney of *Carassius auratus* as a structure with a red tissue colour surrounded by a capsule of connective tissue and a large number of glomeruli.

The cross section of vertebral shows the body of vertebrae that include the centre of vertebrae, neural arches, hemal arches and sphenoid arches, these parts exist in most vertebrates, they provide protection and support for the body and represents a reservoir for calcium and phosphorus [32], the vertebral column consists of a number of vertebrae, each vertebra consisting of a vertebral body and a neural arch that surrounds the spinal cord [33]. Nowroozi et al. [34] showed that the vertebral centre is often similar in size and shape, but the vertebral-associated elements, such as neural arches and sphenoid arches, show regional differences among fish species.

CONCLUSION

In conclusion, the black ghost knifefish *A. albifrons* is characterized by specific type of receptors called tuberous electroreceptors that embedded in the skin, and It has described the structure of some organs tissues in such species for the first time.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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