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Morpho-histological approach of African cat fish (*Clarias gariepinus)* respiratory system with mucocytes and arterial blood supply attribute

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Abstract
This study was designed to precisely clarify the peculiarity of the gills and accessory respiratory organs of *Clarias gariepinus* aimed to find the structural correlations concerning the respiratory area microstructure, mucocyte distribution, and vasculature attribution. Basically, *Clarias gariepinus* have a main and accessory respiratory organ. The main one consists of equilateral triangle gill masses of five pair's gill arches and the accessory one consists of the dendritic organs, Suprabranchial membrane, and the gill fans. The arterio-arterial circulation appeared as extensive vasculature network of afferent and efferent branchial arteries. Histologically, there were matching in the respiratory areas and the covering stratified epithelium with mucocyte at all parts of the respiratory system except at the gill lamellae. The epithelial covering thickness was highest at the gill rakers (106.03±1.94) and lowest at the gill lamellae (14.90±1.10) while mucocyte numbers were highest at the rakers (99.66±2.90) and fewest at the filament (12.83±1.51).

Keywords: *Clarias gariepinus*, respiratory system, mucocytes, arterial circulation.
Abbreviations: *C. gariepinus*, *Clarias gariepinus*; AROs, accessory respiratory organs; ABA, afferent branchial artery; EBA, efferent branchial artery and SBM, suprabranchial membrane.

1. Introduction
Clarias gariepinus as one of the teleost species is massively distributed in Africa and the Middle East owing to the economic value as an affordable source of animal origin protein for different populations [1] It has adversary mechanisms for habitat ecological changes, naturally inhabits rivers, lakes, swamp bottoms, and floodplains and intensely farmed aquaculture species in the Middle East long times ago [2]. The fish is classified as an omnivorous fast-growing bottom feeder, sharp tooth and can feed on agriculture by-products, life and dead animals [3, 4]. Moreover, it is grown in high densities in a less area and has a high conversion rate [5]. Additional peculiarities are; the tolerance of a variety of water parameters changes even by extreme levels; low oxygen tension, high carbon dioxide content even changes in water level dry up in summer with a bimodal respiratory behavior of water and air-breathing even at well-ventilated water and none stress conditions [6, 7]. These abilities related largely to the presence of AROs (dendritic organs, suprabranchial chamber membrane, and gill fans) that enables air-breathing out of water during dryness and during ground walking [8, 9]. The air-breathing ability of different fish was accompanied by circulatory system modification in different ways to lodge blood bi-bath to and from the air-breathing surface. All air-breathing fish excluding lungfishes and snakeheads deficient to lack the ability to separate O2-rich effluent blood of the air-breathing organ from O2-poor systemic venous blood during passage through the central cardiovascular system, which has been understand usually only from anatomical studies [10].

2. Materials and methods
2.1. Animals
A total of twelve African sharp tooth adult male catfish (*C. gariepinus)*, 35-40 cm long, with body mass 1400-1500gm, were used in this study. They were caught live from the River Nile, at Delta region, Egypt, and transported in 100-liter plastic aquaria to the department of anatomy and embryology, Faculty of Veterinary Medicine, Zagazig University, Zagazig,

Egypt. All fish fed on standard fish feed pellets (Grand Fish Feed Company, Cairo, Egypt), kept under observation for two weeks before sample collection and received proper care according to Ethics of Animals Use in Research Committee (EAURC), Zagazig University.

2.2. Macroscopical examination

All fish was anesthetized with 0.01% benzocaine and killed with cervical dislocation then used for anatomical and histological examination [11]. Two fishes were used for branchial and suprabranchial chambers examination and exposing the respiratory organs in situ and photographed by Nikon digital camera and investigated using a dissection microscope (Nikon SMZ-2T; Tokyo, Japan). Two fishes were used for revealing the skeleton of the gill arches. Two fishes were used for gum milk latex injection (red latex) after exposure of the dorsal and ventral aortae and washed by 0.9% saline, then injected with Red (Setacolor™, cardinal red, num. 24, PEBEO, Cedex, France)-colored latex (Rubber latex™, MERCAN, Istanbul, Turkey). The fish were fixed in 10% neutral buffered formalin solution for two days for solidification of the latex solution, before dissection and demonstration of the vascularization of gills and accessory respiratory organs [12]. The nomenclatures in this study were adopted according to Nomina Anatomica Veterinaria [13].

2.3. Microscopical examination

Six fish were used for histological examination where specimens were collected from gills, dendritic organ, suprabranchial membrane, and fans, immediately fixed in Bouin's solution for 48hours. All samples were processed for paraffin embedding technique and tissue segments paraffin blocks were sectioned in 5-micron thickness and stained with hematoxylin and eosin Stain, Alcian blue-PAS and Masson's trichrome [14]. Slides were examined and photographed with Am Scope digital camera-attached Labomed-Digi2 microscope, USA.

2.3.1. Morphometric analysis:

Morphometric analysis of the mucocytes distributions and epithelial thickness at different parts of the main and accessory respiratory organs was performed using six fish. The counting of the mucocytes at different respiratory structures was applied at 40X magnification fields, while epithelial thickness was performed at 10X magnification fields and six fields/each structure/fish were randomly chosen for both parameters. The morphometric analysis was accomplished by one-way analysis of variance (ANOVA), followed by Duncan's multiple ranges Post hoc test for pairwise comparisons using SPSS 25.0 for Windows. The statistical data were expressed as mean ± SE, and the data were considered statistically significant at p values<0.05.

3. Results

3.1. Anatomical findings

3.1.1. Gill anatomy, the *C. gariepinus* gills grossly appeared as two flattened chambers that dorsoventrally attached, each with small ventral aperture. Each chamber was bounded rostrally by mandible, caudoventrally and laterally by an operculum, dorsally by the roof of the oral and pharyngeal cavities and medially in part by the interbranchial septum (Fig.1A, B, C, D).

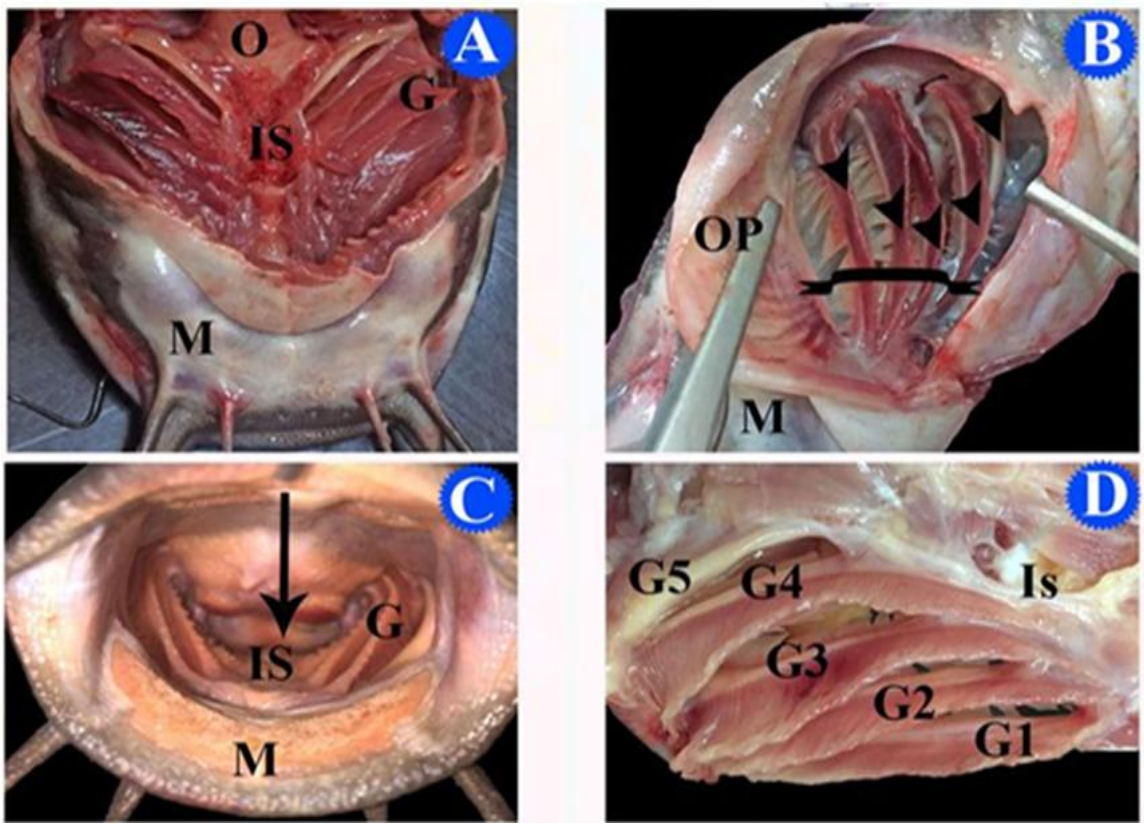

Fig 1: A-C→ photographed of C. *gariepinus* head; **(A)** ventral view with removed operculum and exposed gills (G), Interbranchial septum (Is), Mandible (M) and esophagus (O), and **(B)** ventrolateral view with opened operculum (OP), gill chamber (curved arch), gills (arrowheads) and mandible(M).**C;** anterior view of oral cavity showing gills (G), Interbranchial septum (Is), roof of oropharyngeal cavity(arrow) and Mandible (M). **D-F;** photographed of C. *gariepinus* gills; **(D)** arches ventrolateral view (G1–G5) with Interbranchial septum (IS).

A triangularly shaped gill masses were reflected bilaterally, with a total of five pairs of branchial gill arches. The first four branchial gill arches assembled in the concave border with gill raker and convex one with gill filaments (Fig.1 E). The fifth arch was attached to the pharyngeal floor through its entire length and appeared rudimentary without any gill filaments. The gill rakers come in three parts lateral, medial and middle rows. The lateral and middle rows were represented in all gill arches but the medial was only at the third and fourth ones and rudiment in nodular form projections. Generally, the rakers were partially developed at the first, second and fifth gill arches and well developed at the third and fourth ones (Fig.1 F).

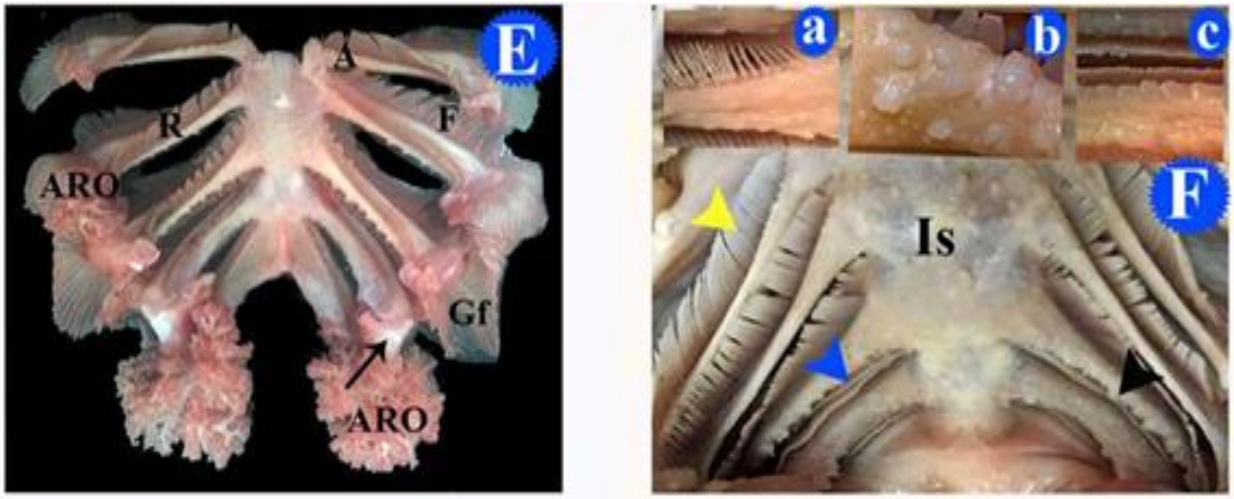

Fig 1: (E) associated dendritic accessory respiratory organ (ARO), gill arch (A), gill filament (F), gill raker (R), gill fan (Gf) and cartilaginous joint (arrow), **(F)** rakers distributed in three directions; lateral (yellow arrowhead), medial (blue arrowhead) and middle (black arrowhead), Gills interbranchial septum (Is), and **Insets;** stereomicrographs of the middle raker of first and second arch (a), third arch (b) and fourth and fifth arch (c).

All arches rostral extremities united and joined medially to the interbranchial septum (Fig.2A). All arches lateral free surfaces boundary by Branchial chamber that separated from the suprabranchial one by the suprabranchial membrane (Fig. 2 B).

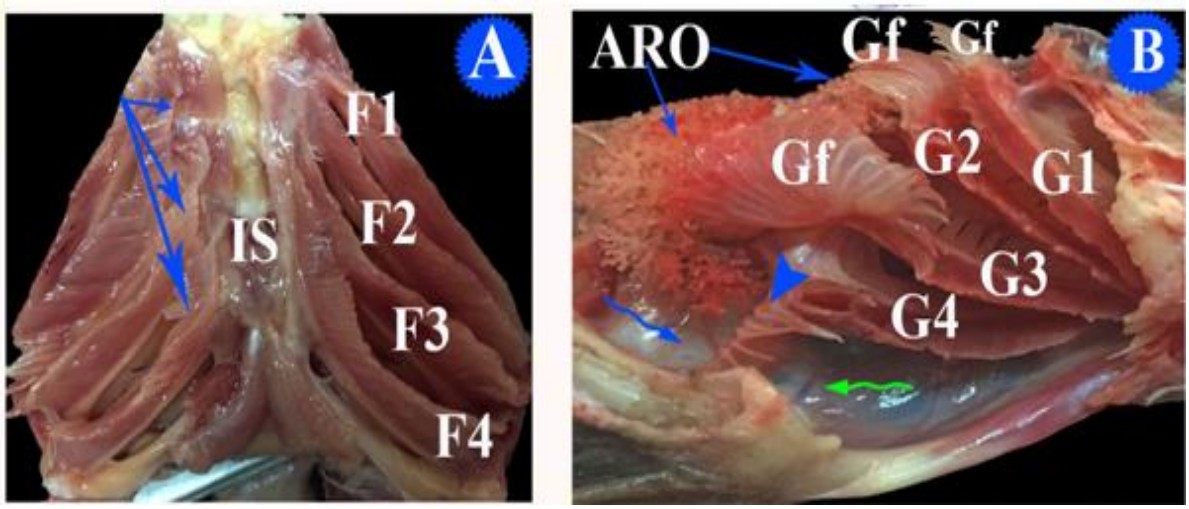

Fig 2A-B; photographed of C. *gariepinus* gills; **(A)** ventral view showed; interbranchial septum (IS) and gill filaments (F1- F4) with rostral extensions of the first four ones (blue arrows), **(B)** lateral view showing gill arches (G1-G4), gill fan (Gf), branchial chamber (green zigzag arrow), suprabranchial chamber (blue zigzag arrow) and serrated border of supra-branchial membrane (arrowhead) with dendritic organs (ARO).

The gill filaments per each holobranch were arranged in two medial and lateral hemibranchs at the first four gill arches that varied in length in-between and inside the same arch. The filaments had overlapped rostral extensions and free caudal borders (Fig.2 C). The skeleton of the gill arches arranged in five segments from medial to lateral; the basibranchial (BB 2-4), hypobranchial (HB 1-4), ceratobranchial (CB 1-5), epibranchial (EH 1-5), and pharyngobranchial (PB 3-4) respectively (Fig.2D).

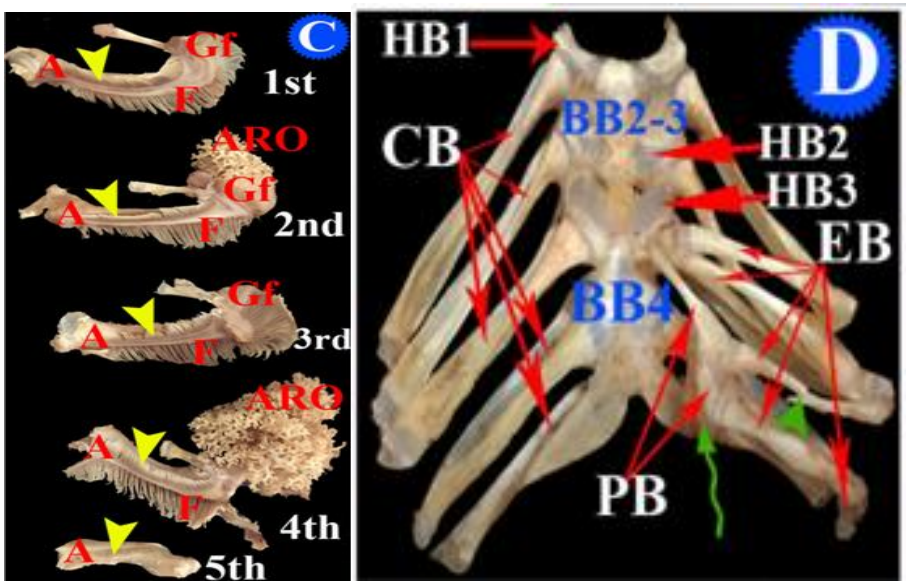

Fig 2: (C) arches in order (1st -5th) and its associated dendritic organ (ARO), gill fans (Gf), gill raker (arrow heads) and gill filament (F), **(D)** arches skeleton dorsal view with basibranchial (BB2-3), (BB4), hypobranchials (HB1,2,3), ceratobranchials (CB), epibranchials (EB), pharyngobranchials (PB), tooth plate (zigzag arrow) and uncinate process (arrow head)

3.1.2. Accessory respiratory organs

3.1.2.1 The suprabranchial chamber had two apertures inhalant and exhalant, and by dissection, it reflected a grown-up arborization came from both sides of the second and fourth-gill arches, dendritic organs (Fig.2E).

3.1.2.2 Gill fans and suprabranchial membrane, the skull attached highly vascular suprabranchial membrane (respiratory membrane) and gill fans that guarded the chamber opening. The fans formed by modified medial hemi branch of the first two gill arches and the lateral hemi branch of the third, into elaborated interconnected rays with serrated terminal free ends (Fig. 2 C&F).

3.1.2.3 The dendritic organs configured two pairs of dendritic form structures; small structure (rostral part) attached the second-gill arch and the other is larger (rostral part) and attached the fourth-gill arch (Fig.2G). The small structure had the main stem that further divided into two secondaries. The large part main stem had five to six subbranches. Then all sub-branches of both small and large structure were successively divided and finally ended by knob-like structures bulbous terminals with cartilaginous cores. The whole skeleton of the dendritic organs was cartilaginous at all its structural levels and supported by arcus brachialis muscle at the fourth-gill arch.

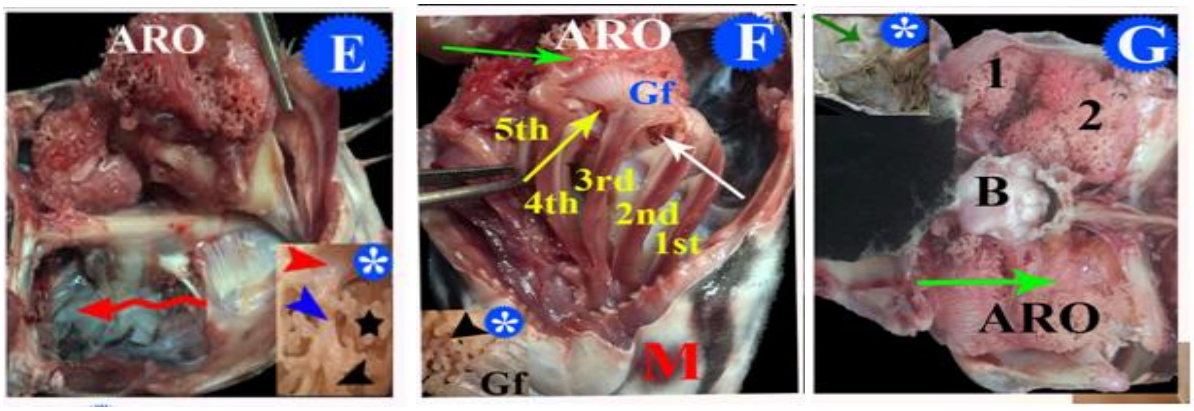

Fig 2.E-G: photographed of C. *gariepinus* dendritic organ (ARO);**(E)** relation to the suprabranchial chamber (zigzag arrow), **Inset;** (*) stereomicrograph showed organ primary stem (red arrowhead), secondary branches (blue arrowhead), tertiary branches (black arrowhead) and bulbous terminals (star), **(F)** relation to gill arches (1st -5th) ventrolateral apertures; first aperture (white arrow), second aperture (yellow arrow), suprabranchial membrane (green arrow), gill fan (Gf) and mandible (M), **Inset;** (*) stereomicrograph of the bulbous terminals (arrowhead) and gill fan (Gf), **(G)** viewed from dorsal aspect of the head with its rostral part(1), caudal part (2), suprabranchial membrane (arrow) and the brain (B), and **Inset;(*)** stereomicrograph of suprabranchial membrane (arrow).

3.1.3. Arterial supply of the respiratory system

C. gariepinus respiratory system supplied mainly by dorsal and ventral aorta, following up the arterial blood supply distribution to the system, there are four pairs of afferent branchial arteries supplied the gill arches alternatively. The first one originated from the bulbous arteriosus and the other three from the ventral aorta (Fig.3A). After the afferent branchial arteries (ABA) penetrate the arches tissues, they subdivided into recurrent and concurrent branches. The recurrent branches provided the anterior parts of the arches while the posterior of the arches supplies by the concurrent branches. The concurrent ones also supplied the second and the fourth arches and accessory respiratory organs. Along the course of the afferent branchial arteries they emerge

subbranches to the gill filaments, the filamental arteries passes as the afferent filamental artery that by itself passed afferent lamellar arterioles. (Fig.3B). The latter collected into the lamellar sinus then extended as efferent lamellar arterioles that collected into the efferent filamental artery of the corresponding arch (Fig. 3 C). The efferent branchial arteries (EBA) were the same number and arrangement of ABA.

The dendritic organs were provided by afferent and efferent branchial arteries from their corresponding gill-arches (Fig. 3 D). Then these arteries re-branched within the organ with the same distribution of the blood vessel of gill filament. The supra-branchial membrane blood supply found to arise from three groups of blood vessels that originated from; the first two EBA, lateral aorta and dorsal aorta. The whole three groups branched and re-branched in an extensive manner to cover the whole surface of the membrane (Fig.3E, F).

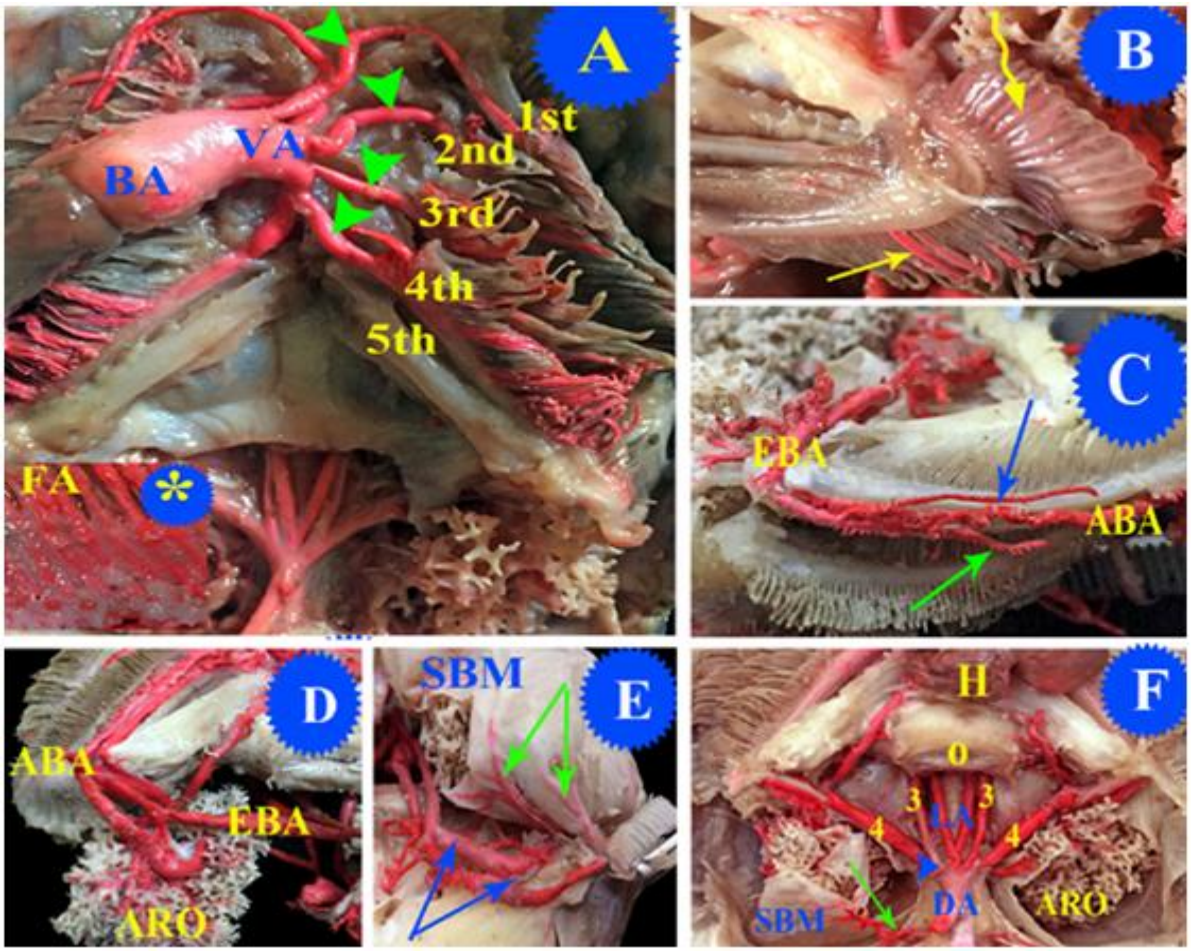

Fig 3: A; Photographed of C. *gariepinus* of the ventral view of the heart showing bulbus arteriosus (BA), ventral aorta (VA), afferent branchial arteries(arrowheads) and gill arches (1st - 5th). Inset; (*) stereomicrograph of the filamental arteries (FA).**B-C;** photographed of C. *gariepinus* arterial side blood vessels of the respiratory organs**;(B)** Afferent branchial arteries that give the filamental arteries to ceratobranchial part (arrow) and gill fans of the epibranchial part (zigzag arrow), **(C)**Efferent branchial arteries (EBA), formed by the pre-lamellar (blue arrow) and post-lamellar (green arrow) arterioles of filamental arteries of afferent branchial arteries (ABA), **(D)** dendritic organ (ARO) supplied by afferent branchial arteries (ABA) and efferent branchial arteries (EBA),**(E)**The suprabranchial membrane (SBM) blood supply arise from the first two EBA (blue arrows) cranial branches (green arrows), **(F)** The suprabranchial membrane (SBM) blood supply arises from; middle arteries (green arrow) of lateral aorta (LA), dorsal aorta (DA), and common trunk of third and fourth efferent branchial arteries (arrowhead). The whole efferent branchial arteries showed in number from (1-4).

3.2 Histological findings

3.2.1. Gills, The *Clarias gariepinus* gill's microscopic details appeared with the basic structure of gill arch or brachial arch, gill rakers and gill filaments. Each arch had two borders; rakers carried on concave border and filaments carried on convex one. The covering epithelium of the brachial arches at their concave aspect expanded by bony plates of variable lengths that varied according to the number of the arch into; long, short and nodular plates or gill rakers (Fig.4A, B&C).The PAS-positive cells mucocytes nearly covered most of the surfaces of processes of the long and short form rakers while at the nodular type the mucocytes were concentrated in patchy aggregation. At the medial aspect of the gill arches-rakers and inter-rakers areas emergeda markedly thickened covering epithelium that comes unique, of enormous numbered mucocytes interrupted by some gland apertures and taste buds (Fig.4D). At the longitudinal section of the brachial arch, the convex surface displayed the gill filament with triangular support partially ossified cartilaginous plates, brachial rays that possessed as a thin process toward the efferent edge of each filament. The thin process varied in length and could reach the two-thirds of the filament length. The brachial rays were in conjunction with the abductor muscles of the gill arch. While The terminal free end of the gill filament arrived at the dilated core with engorged blood vessel sinusoids (Fig.4 E, F&G).A uniform stratified epithelium mostly of squamous pavement cell type covering; the convex surfaces of the gill arches; gill filaments, gill lamellae and the interlamellar spaces the main difference was at the number of cell layers. The gill filament revealed

increased number of cell layers than the gill lamellae. The filaments showed more thickness towered its afferent and efferent ends. At all level of gill arch substructure, the epithelial pavement cells occupied most of the covering areas, undifferentiated basal cells placed at the basal layer and intermediate cells at the middle. The gill filaments showed bilateral folds transversely raised in the form of secondary lamellae (the respiratory area) with the same type of covering epithelium. The lamellar core evidenced to be highly vascularized as the presence of enormous pillar cells covered by a basement membrane and two epithelial cell layers. The pillar cells had central cell bodies and flanges at the cell sides. The adjacent pillar cell flanges encountered lacunar blood spaces under the layers of the covering epithelial (Fig.4H). The basement membrane between the pillar cells and the epithelium appeared as PAS-positive columns transversely across the lamellae and come to continue with that of the basal membrane of the gill filament. Mucocytes were distributed along the filament and more concentrated towered the extreme edges, while the chloride cells mostly found at the origin of the secondary lamella in few numbers (ionoregulatory cells). Secondary lamellae appeared devoid from any mucocytes (Fig.4 I &J). The afferent and efferent filamental arteries were clearly visible at the efferent edges of the filament while the Central venous sinus (CVS) was mostly noticed under the bases of the lamellae. In addition to subepithelial dense nervous network within the filament core. Medially, where the branchial arches rostral extremities united and joined to the interbranchial septum, the cross-sectional areas revealed covering epithelium of stratified type too, with mucocytes and corecontent of; connective tissue fibers, muscle bundles, plates of elastic cartilage, adipose tissue and blood vessels (Fig.4K&L).

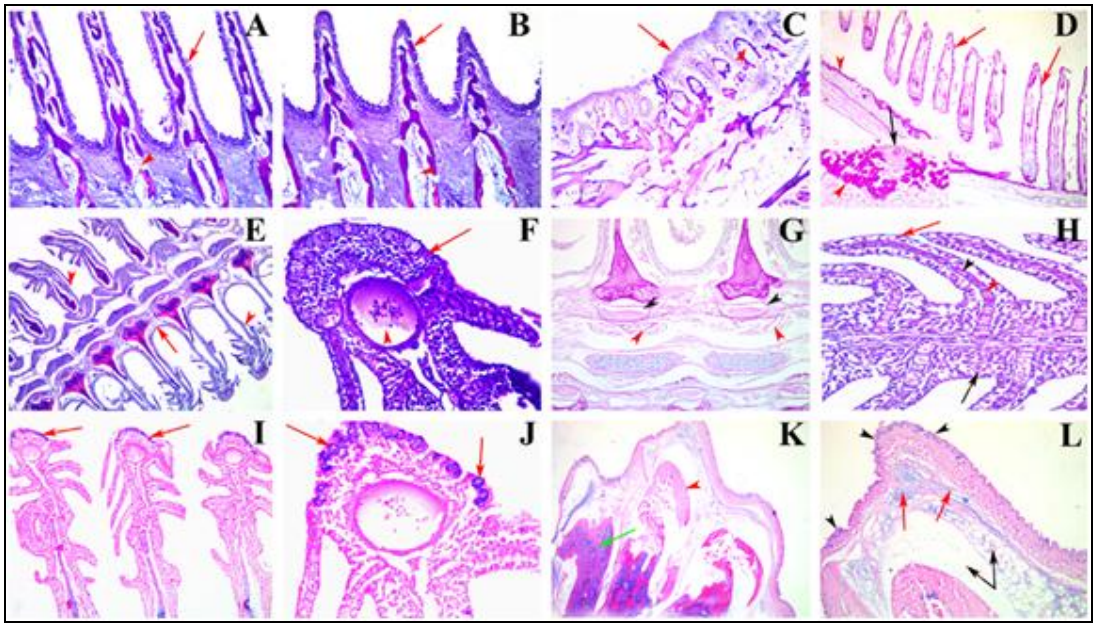

Fig.4: A-D; photomicrograph of C. *gariepinus* gill rackers; **(A)** long type **(B),** short type and **(C)** nodular one; all with bony plates of variable lengths, rich vasculature (arrowheads), covering stratified epithelium (arrows) (H&E stain X10), **(D)** PAS positive mucocytes distribution at the long type rakers (red arrows) and inter raker (red arrowheads). Inset: Patchy aggregation of mucocytes at the inter rakers areas (red arrowhead) with gland aperture (black arrow) (PAS. X4, inset: X40). **E;** brachial arch showing; arch convex surface (arrow) with gill filament (arrowheads) (H&E X4). **F-I;** photomicrograph of C. *gariepinus* gill filament; **(F)** efferent edges with thickened covering epithelium (arrow) and highly vascularized core lacunar blood spaces (arrowhead) (H&E stain X40), **(G)** afferent edge with filament basal central venous sinus (black arrowheads) and gill arch abductor muscles (red arrowheads) (PAS.X10), **(H)** gill filament (black arrow) with secondary lamellae (red arrow), lamellar core lacunar blood spaces (black arrowhead) with pillar cells (red arrowhead) (H&E stain X40), **(I)** gill filament efferent edges with surface mucocytes distributions (arrow) (AB.X10), **(J)** High magnification of **Fig**. **I**, showing AB positive mucocytes (arrows)with dark blue mucus content (AB.X40).**K-L;** photomicrograph of *C. gariepinus* brachial arch; **(K)** origin with core cartilage (arrow), muscle bundles (arrowhead) and fat cells (F), (AB.X4), and **(L)** brachial arch with; numerous mucocytes covering epithelium (arrowheads), sub-epithelium connective tissue (red arrows) and fat cells (black arrow). (AB.X4).

3.2.2 Accessory respiratory organs

3.2.2.1 Gill fans and suprabranchial membrane: Towards the apex of the first, second and third branchial arches, the gill filaments microscopically appeared; gradually fused and covered with thickened continuous sheets of the stratified epithelial type forming the gill fans. In which the core content of the basal part of the fans displayed partially ossified cartilaginous plates. These plates became bifurcated upward enclosing the peripheral blood capillary of the corresponding filaments inside (Fig.5A&B). Consequently, the core tissue elements became corrupted with the disappearance of the cartilaginous part and widen blood spaces clearly displayed. Continuously, regular arranged connective tissue fibers and muscle bundles with high vasculature upwardly extended and all quite covered with the same stratified epithelium. Toward the fan free border, the core tissue become occupied with extensive connective tissue fibers and bundles that mostly appeared with Masson's trichrome positive reaction collagen type (Fig. 5C&D). Further folding of the covering epithelium gave the fans more corrugated surfaces. Mucocytes were distributed in large numbers at the fans covering epithelium and at the areas of the epithelium infoldings (Fig. 5E). The suprabranchial membrane showed stratified covering epithelium with mucocytes, the same as the dendritic organ. Additionally, the membrane also matching the organ in the presence of the transverse capillaries that emerged from afferent blood vessels and run to the surface epithelium cell layers forming respiratory lamellae (respiratory area) (Fig. 5F). Fig 5

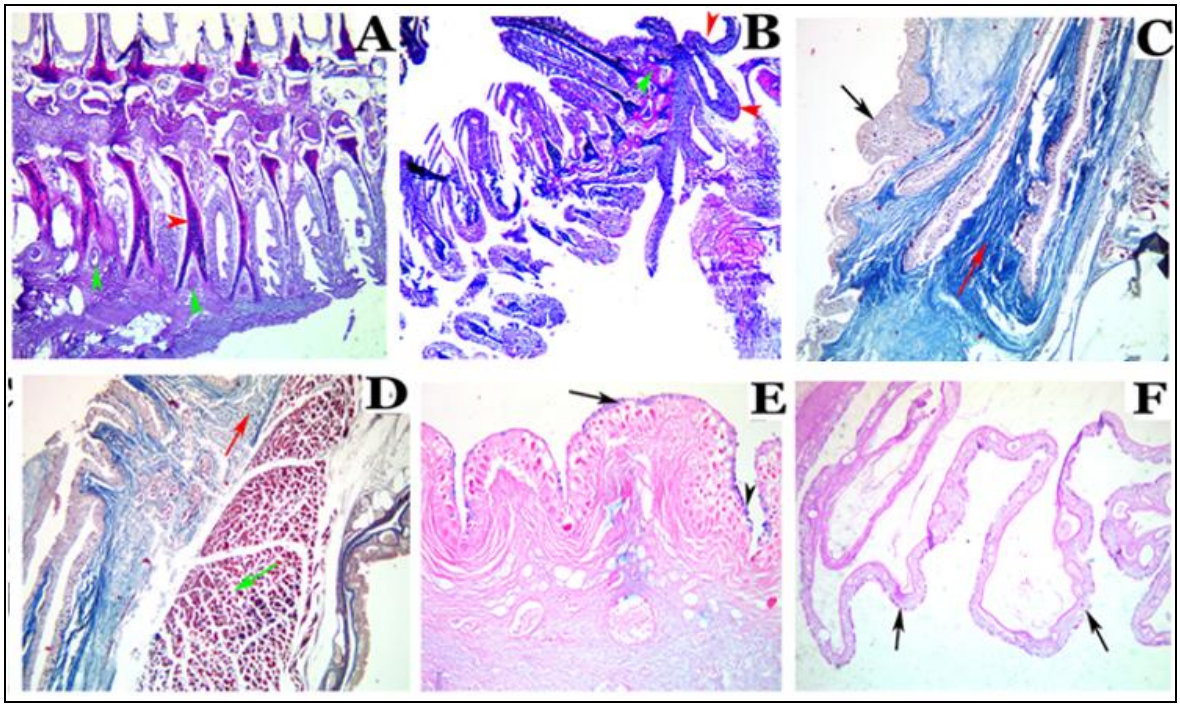

Fig 5:A; Photomicrograph of C. *gariepinus* gill fan at the second branchial arch;**(A)** with gill filaments fusion at its origin; partially ossified bifurcated plates (red arrowhead) enclosing the peripheral blood capillary of the original filament tissue core (green arrowhead),(H&E.X4),**(B)** gradual extension of the covering epithelium (red arrowhead) over the fused gill filament with ossified tissue core (green arrowhead),(H&E.X4), **(C)**free border with extensive content of fibrous collagen bundles (red arrow) and thick stratified covering epithelium (black arrow), (Masson's trichrome stain X4), **(D)** basal part muscle bundles (green arrow) and connective tissue (red arrow) core, (Masson's trichrome stain X4), and (E) covering epithelium with numerous mucocytes at exposed surface (arrow) and at the surface infoldings too (arrowhead),(PAS. X.40). F-Photomicrograph of *C. gariepinus* suprabranchial membrane stratified covering epithelial with mucocytes (arrows), (H&E.x4).

3.2.2.2 dendritic organ: It originated from the posterior extremity of the second and fourth-gill arch. It had enriched blood vasculature core and elastic cartilage content at all substructure levels of the organ; the main stem, articulating sites, primary stem, successive branch stem, terminal branch stem and bulbus terminals (Fig.6A, B, C, D&E). Fig 6.

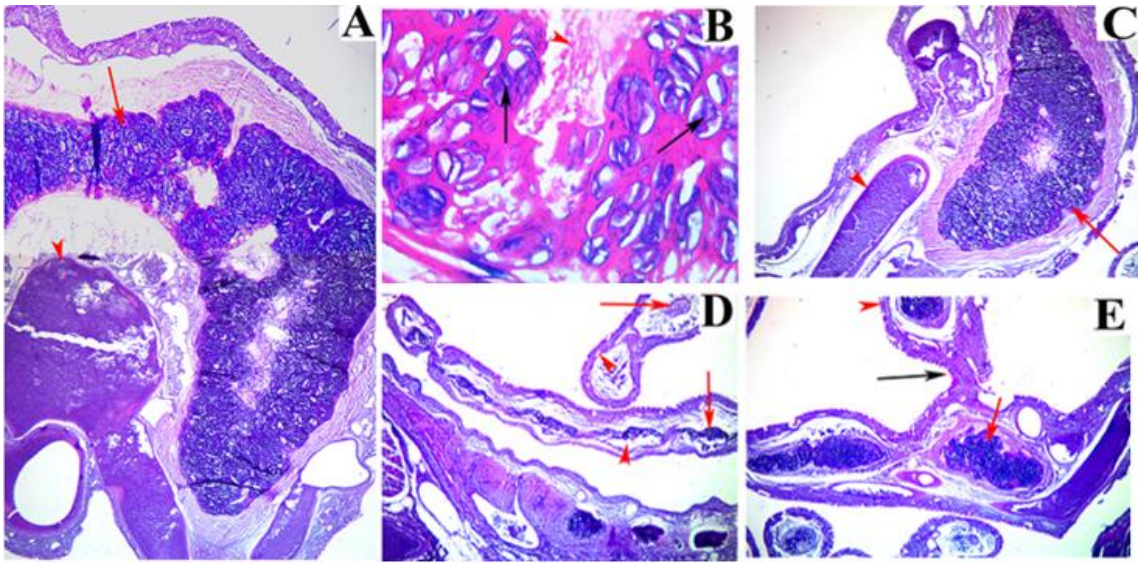

Fig 6: A-E; Photomicrograph of C. *gariepinus* dendritic organ, originated from the posterior extremity of the fourth gill arch, with core of elastic cartilage (arrows) and blood vasculature (arrowhead); (A) Main stem, (C) primary stem, (D); successive branch stem, (E) terminal branch stem; (H&E.X4), and (B) Main stem (AB.X40).

Histological findings revealed the dendritic organ substructures with a covering epithelium of stratified type with mucocytes at the organ main stem, terminal knobs, successive branches and at the articulating sites too. The core was occupied with elastic cartilage, connective tissue, blood vasculature, lymphatic drainage in addition to muscle bundles only at the main stems (Fig. 7A&E). The dendritic organ had transverse blood capillaries emerged from afferent blood vessels and run to the surface epithelium cell layers forming respiratory lamellae(respiratory area). The respiratory lamellae found as paired rows of epithelial infoldings with narrow interval spaces. The transverse capillaries that covered by that epithelium appeared to arrange in parallel columns and the blood channels matched that of the gill secondary

lamellae and in the distribution of the pillar cell too (Fig. 7 B&F). Large numbers of mucocytes were distributed at all parts of the dendritic organ (Fig. 7 C, G, D & H). But, the best-seen blood channels and most numerous mucocytes were at the terminal stems. Fig 7.

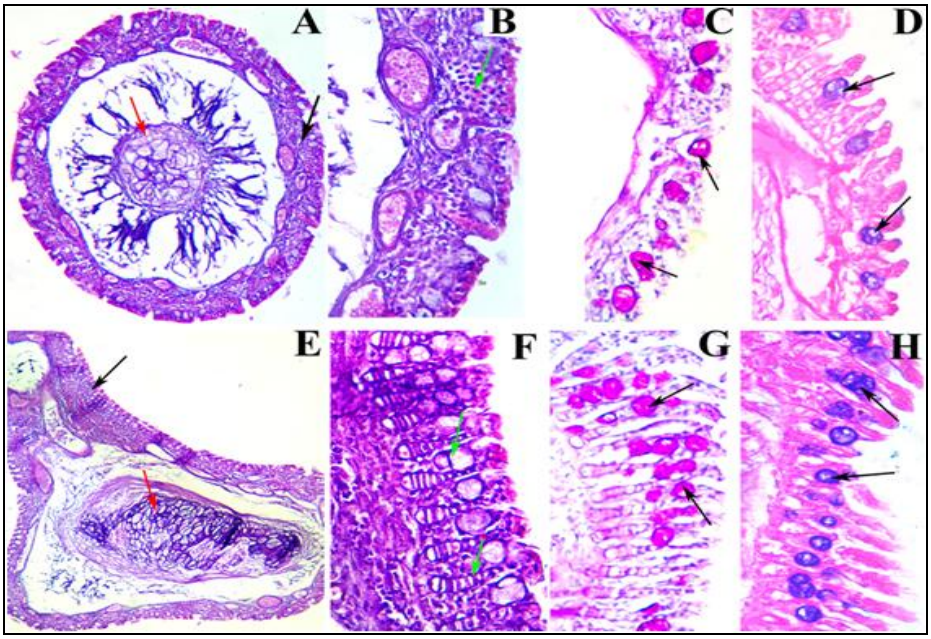

Fig 7: Photomicrograph of C. *gariepinus* dendritic organ bulbus terminals (A) and terminal branch stem (E); with a core of elastic cartilage (red arrows) and stratified covering epithelium (black arrows). Consequently (A&E) high magnification showed: transverse capillaries of parallel columns channels (arrows) (B&F), (C&G): PAS positive mucocytes (arrows) and (D&H): AB positive mucocytes (arrows) (A,B,E,F:H&E.X.4&10) (C,G:PAS and D,H: AB X.40).

3.2.3Morphometric analysis

3.2.3.1. The epithelial thickness of the gill substructures; arch, arch process, arch internal parts, filament main parts, filament terminal ends, and lamellae were summarized in tables (1&2). It was clear that there are no significant differences in the epithelial thickness of these structures among the four branchial arches, but the significant differences were in-between the different structures of each branchial arch. The epithelial covering thickness was of the highest value at the gill racker (106.03 ± 1.94 µm) and lowest at the gill lamellae (14.90 ± 1.10 µm).

Table 1: Epithelial thickness of gills different parts. The values in all tables are expressed as means ± SE. (n=6).Groups indicated with different superscript letters in the same line are statistically significant (p<0.05).

ARO-Epithelial thickness (µm)					
	Dendritic organ			Fans	Suprabranchial membrane
	Bulbous end	Main stem	Sub-branches		
Large dendritic organ	47.43 ± 1.56^{a}	61.40 ± 0.79^{b}	71.16 ± 0.94^{c}	74.83 ± 1.12^{c}	62.66 ± 1.88^{b}
Small dendritic organ	49.16 ± 0.96^{a}	60.88 ± 0.96^{b}	72.33 ± 1.06^{c}	73.41 ± 1.06^{c}	64.16 ± 0.79^{b}

Table 2: Epithelial thickness of ARO different parts.

Gill epithelial thickness (µm)						
Gill arch	Arch (µm)	Raker Process	Raker interstitial parts (µm)	Filament Main part	Filament terminal end	Lamellae
First	104.60 ± 2.60^{a}	28.46 ± 0.93^{b}	51.96 ± 0.84^{c}	42.13 ± 1.07^{d}	53.36 ± 1.47^{c}	14.90 ± 1.10^{e}
Second	103.83 ± 2.29^{a}	29.74 ± 0.98^{b}	52.46 ± 0.64^{c}	44.24 ± 0.48^{d}	56.41 ± 0.98^{c}	15.96 ± 0.93^{e}
Third	105.16 ± 1.14^{a}	28.66 ± 1.09^{b}	54.83 ± 0.84^{c}	43.33 ± 0.75^{d}	54.83 ± 1.08^{c}	16.33 ± 1.28^{e}
Fourth	106.03 ± 1.94^{a}	28.33 ± 0.93^{b}	55.16 ± 0.72^{c}	42.33 ± 0.71^{d}	55.33 ± 1.16^{c}	16.66 ± 0.28^{b}

3.2.3.2. Mucocytes numbers were shown in tables (3 & 4) the highest mucocytes number was at the gill racker (99.66 ± 2.90) and fewest at the gill filament (12.83 ± 1.51). It was intersecting that mucocytes were completely absent at the gill secondary lamella.

Table 3: Mucocytes number at gill different parts.

	Gill mucocytes (mean ± SE)					
	Arch	Raker Process	Raker interstitial parts	Filament Main part	Filament terminal end	Lamellae
1st gill arch	20.50 ± 1.43^{a}	30.66 ± 0.71^{b}	99.66 ± 2.90^{c}	12.83 ± 1.51^{d}	27.83 ± 1.44^{b}	0.00
2nd gill arch	20.33 ± 0.91^{a}	30.83 ± 1.13^{b}	97.50 ± 2.36^{c}	16.16 ± 0.83^{d}	24.83 ± 1.72^{e}	0.00
3rd gill arch	21.00 ± 1.29^{a}	31.83 ± 0.79^{b}	99.33 ± 2.24^{c}	14.00 ± 1.75^{d}	27.33 ± 1.28^{e}	0.00
4th gill arch	20.83 ± 0.94^{a}	30.50 ± 0.76^{b}	97.00 ± 1.82^{c}	15.33 ± 1.52^{d}	28.00 ± 2.00^{b}	0.00

Table 4: Mucocytes number at ARO different parts.

ARO mucocytes (mean ± SE)				
Number of mucocytes	**Dendritic organ mucocytes**			**Fans**
Large dendritic organ	**Bulbous end**	**Main stem**	**Sub-branches**	
	17.66 ± 1.38[a]	30.00 ± 1.98[b]	24.66 ± 1.62[c]	15.16 ± 1.44[a]
small dendritic organ	18.33 ± 0.88[a]	27.33 ± 2.17[b]	26.33 ± 2.10[c]	14.66 ± 1.40[a]

4. Discussion

It has been years since the anatomical investigators recorded *Clarias garipeinus* respiratory system as a gill system, accessory respiratory organs and categorized it as air-breathing fish [15]. The gills in catfish were present in two interconnected dorsoventral ly flattened gill chambers, that had a small caudoventral opercular opening in contrast to the wide ventrolateral opening of tilapia [15] and Grey Gurnard and Striped Red Mullet Fish [16]. On the other hand [17] revealed a single caudal opercular opening in teleosts. Regarding our results, the anatomical structure of the gill Clarias gariepinus was similar to other teleosts. Although the variable number of gill arches between the different teleosts; the four pairs of gills was the widespread number in most fishes [18, 19, 15; 20, 21, 1, 16]. Our results disagreement with [22] in common carp that revealed the fifth pair united forming the pharyngeal bone. On the other hand [16] in the striped red mullet recorded that only three pairs of gills were present. In agreement with the previous literature that the shape, number and density of rakers on arches was a good indication of the feeding habits of fish and the 5th-gill arch contained lateral rakers only [1, 15, 23, 24]. On the same line with [15, 18] in Clarias gariepinus, the present investigation revealed that the gill filaments of the epibranchial part of the first three gill arches were modified into elongated interconnected rays forming the gill fans which considered one of the air-breathing organs. Our results agreed with [8, 15, 25] they explained that the arborescent organ was connected by a cartilaginous joint with the posterior extremity of the second and fourth gill-arches. On contrast to [26] that recorded that, the labyrinth organ was attached to the 1st-gill arch only. Regarding the architecture of the arborescent organ was coordinate well with the findings of [25] but there were differences in the numbers of secondary branches originating from the primary stem.

The arterio-arterial pathway of the respiratory organs in *Clarias batrachus* arose from dorsal and multiple ventral aortas which were the typical vascular pathways of teleosts [27]. The afferent branchial arteries (ABA) were four arteries only due to the absence of ABA of the fifth-gill arch. The blood passed from the afferent branchial arteries to the filamentous arteries that collected blood from pre lamellar and post lamellar ones. the filamentous arteries rejoined to form efferent arteries of the same arch [28, 29]. The first and second efferent branchial arteries united to form a common trunk. The efferent branchial arteries rejoin from both sides to form the median dorsal aorta. The dorsal aorta recorded as a major artery that carried oxygenated blood from the efferent branchial arteries to the branches that supply the body organs [30]. The first and second efferent branchial arteries were passed separately [30], which disagreement with our results. The dendritic organs were provided by parallel blood supplies to their corresponding gill-arches afferent and efferent branchial arteries. Then these arteries re-branched within the organ to adapt its architecture. Parallel blood vessel to gill filaments was distributed at the dendritic organ [26]. The supra-branchial membrane blood supply found to arise from three groups of blood vessels that originated from; the first two EBA, lateral aorta and main aorta. The whole three groups branched and re-branched in an extensive manner to cover the whole surface of the membrane [27]. No anatomical modification observed in the arterial blood supply of gills or any of the AROs. And the arterial blood supply of the dendritic organ was parallel with that of the gills at all its level. Ventral aorta as had the upper hand in arterial blood supplying of gills and all AROs of *C. gariepinus*.

Microscopically, the fans covered by the thickest stratified cuboidal epithelium found in all the studied respiratory parts with characteristic large spheroid mucocytes. The microstructural tissue characteristics of the secondary lamellae (high vasculature blood spaces with pillar cells and thin covering epithelium and almost absence of mucocytes) facilitate their function as the basic respiratory unites of the gills through increasing the respiratory area of the gill filaments [17]. The adjacent lamellae proximities are completing the griddle like ordering of the filaments leaving a space in between the lateral wall of the interbranchial septum and the lamellae edges. Thus, the water flow behind the lines of the lamellae on and out forming water channels directions counter the lamellar blood flow [31].

The dendritic organ was noticed as a tree-like structure with numerous bulbus ends that articulating with the posterior extremities of the second and fourth gill-arches [16, 25].

The facultative air-breathing ability of c g related to the presence of ARO and the suprabranchial membrane [32]. The ARO had dichotomous branching of stems and terminal bulbous ends that had a core of connective tissue and elastic cartilage covered by highly vascularized stratified epithelium with large rounded mucous cells. This enriched vascularity arose from the afferent blood vessels which branched to the transverse capillaries of the vascular papillae at the free surface epithelium of the ARO in the form of parallel columns. The suprabranchial membrane had a matched vascular structure as seen in the ARO. The epithelial cover and their vasculature form the main surface respiratory areas at both accessory organs [32]. The core had a unique feature of vascularized cartilage via invaded blood vessels. These features allow articulation and movement of the ARO. Microscopical Similarity of gills Secondary lamellae, ARO, and suprabranchial membrane transverse capillary blood channels. These channels separated by pillar cells and the pillar cells separated from the epithelium by a basement membrane. This similarity emphasis the structural matching of the surface respiratory areas at different parts of the respiratory system of that fish [33].

The highest epithelial thickness (106.03 ± 1.94 μm) that recorded on the branchial arch might have a protective function while the lowest epithelial thickness (14.90 ± 1.10 μm) at the gill secondary lamellae might facilitate the process of gases exchanges. The highest percentage of the mucocytes (99.66 ± 2.90%) was at the gill rakers near the taste buds could help in removing the food sediment from taste buds' surfaces. This allowing continuous distinguishing sense of different stimuli, as the raker associated the entrance of the pharynx [31].

From all the above, the catfish respiratory organs come clear with structural-functional specialization rather than the animal's lungs. The ventilation mechanism in gills is unidirectional and continuous while the lungs were always tidal. The most significant functional difference is that part of the lung content does not change during every single ventilation cycle while the gills not. Although the portion of unrenewed lung content serves as a buffer between the blood and the outside medium and the main lung function associated with carbon dioxide pressure, gills have the same risk of washing up ions and over ventilation in spit its role in the osmoregulation, due to uncontrollable interlamellar water flow [33, 18].

5. Conclusion

This study reflected a correlation of matched respiratory areas microstructure at the main and accessory respiratory system. These areas possessed extensive vasculature at; gill secondary lamellae, dendritic organ and suprabranchial membrane with extensive transverse capillary blood channels. The highest epithelial thickness was recorded at the branchial arch might have a protective function while the lowest epithelial thickness was at the gill secondary lamellae might facilitate the process of gases exchanges. Mucocytes number was highest at the gill racker epithelium and fewest at the gill filament that could be functionally adapted.

6. References

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