Structure of the kidney in the coelacanth *Latimeria chalumnae* with reference to osmoregulation

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The morphology of the nephrons of the coelacanth *Latimeria chalumnae* was investigated by light microscopy. Each nephron is composed of a large renal corpuscle with well-vascularized glomerulus, non-ciliated neck segment, proximal convoluted tubule divided into distinct first and second segments, non-ciliated intermediate segment, distal tubule, collecting tubule and collecting duct. The parietal layer of the Bowman’s capsule of the renal corpuscle is composed of low cuboidal cells. The short non-ciliated neck segment is lined by cuboidal epithelium. The first and second proximal segments display a prominent brush border and contain amorphous material in their lumen. The second proximal segment differs from the first segment in having taller columnar epithelium and a relatively narrow lumen. The intermediate segment is lined by non-ciliated columnar epithelium and its lumen appears empty. The distal tubule is narrow in diameter and its cuboidal epithelium is devoid of intercalated cells. A unique feature of *L. chalumnae* is having binucleate cells in the tubule and collecting duct epithelium. The renal arteries have poorly developed tunica media and its cells contain granular material. The structure of *L. chalumnae* nephrons correlates well with their osmoregulatory function and resembles those of euryhaline teleosts.

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Key words: binucleate cells; collecting ducts; neck segment; renal corpuscles; renal tubules.

INTRODUCTION

The coelacanth, *Latimeria chalumnae* Smith being the only representative of the crossopterygian (lobe-finned) fishes considered to be the ancestor of the land vertebrates, occupies a unique position in vertebrate phylogeny (Romer, 1966). It is considered a living fossil since it has survived over 350 million years with few, if any, morphological changes (Thomson, 1991; Ward, 1992; Gorr & Kleinschmidt, 1993). Since *L. chalumnae* is a living link from the ancient vertebrates, investigating structure and function of its tissues could be of significant evolutionary interest.

In *L. chalumnae*, the anterior kidneys are absent. The two kidneys are closely approximated into a postero-median renal mass (Millot & Anthony, 1973a). The kidneys in other vertebrates are located dorsally in the body cavity (Romer & Parsons, 1973b).
1985). In sharp contrast, in *L. chalumnae*, the non-lobate kidneys that are of the usual mesonephric type occupy a unique position in being ventral and post-anal, resting on the ventral musculature and dorsally in contact with the visceral mass. According to Millot & Anthony (1973a, b), this displacement in kidney position has been induced because of the presence of a large, thin walled, fat-filled gas bladder or degenerate lung. The cloaca, presumably a primitive vertebrate feature, is absent in both sexes of *L. chalumnae*. The two mesonephric ducts are situated on the dorsal surface of the kidneys and open separately into the double urinary bladder. In the male, the urethrae open into the rectum, but in the female they open directly to the outside, posterior to the anus (Dingerkrus *et al.*., 1978).

Renal morphology of different fishes living in different environments of varied salinities were well described by Hickman & Trump (1969), Hentschel & Elger (1989) and reviewed by Elger *et al.* (2000). The structure of the opisthonephric kidney of the sea lamprey *Petromyzon marinus* L. was studied in detail by Youson & McMillan (1970a, b, 1971a, b). The morphology of the nephrons of freshwater rainbow trout *Oncorhynchus mykiss* (Walbaum) was investigated by Anderson & Loewen (1975). Studies have been carried out on the kidney of elasmobranchs, spurdogfish *Squalus acanthias* L. and little skate *Raja erinacea* (Mitchell) by Lacy & Reale (1985a, b, 1991) and Lacy *et al.* (1987), sturgeon-kidney by Ojeda *et al.* (2003) and recently on the kidney of African lungfish *Protopterus dolloi* Boulenger by Ojeda *et al.* (2006).

The first morphological study of the excretory system of *L. chalumnae* was made by Millot & Anthony (1973a). Since then, no work has been done on *L. chalumnae* kidney. This may be due to great difficulty in obtaining well-fixed tissue of this extremely rare animal.

This study was undertaken to confirm and extend the observations of Millot & Anthony (1973a) on the structure of the mesonephric kidney of *L. chalumnae* and to correlate its structure with osmoregulatory function.

**MATERIALS AND METHODS**

The mesonephric kidneys were harvested from an 86 cm immature female specimen of *L. chalumnae* captured alive in the Indian Ocean off Grand Comoro Island during the International Expedition of 1972. The kidneys were fixed by immersion in an iso-osmolar glutaraldehyde solution (Lagios, 1974). A complete transverse piece of the preserved kidneys was obtained and post-fixed in 2% osmium tetroxide in 0-1 M phosphate buffer (pH 7.4) and dehydrated in ethanol series. A large portion of the dehydrated renal mass was cleared in xylene and embedded in paraplast (melting point 57° C). The blocks of this tissue were sectioned transversely at 6 or 8 μm and stained with Harris haematoxylin–eosin. The remaining portion of the dehydrated piece was treated with propylene cyanotrile and embedded in polyBed 812 resin (Polysciences; www.polysciences.com). Polymerization was carried out at 60° C overnight. Semi-thin sections (1 μm) from several blocks of the tissue were cut on a Porter-Blum MT-2 ultramicrotome (http://www.m410.net/Lab-equipment/microtomes) and stained with azure II and toluidine blue stains. All sections were studied and photographed with an American optical series 20 light microscope (www.microscopesfromnightingale.com).

The different regions of the nephrons of a female *L. chalumnae* were identified by comparing them with urinary tubule segments of fishes and higher vertebrates. The measurements of different parts of the nephrons were obtained from the photomicrographs. Photomicrographs obtained from the resin-embedded tissue showed less shrinkage and higher resolution.
in comparison with paraffin-embedded tissue. Unfortunately, the fixation of the tissue was not suitable for electron microscopic study.

RESULTS

In this juvenile female *L. chalumnae*, each kidney contains numerous nephrons and collecting duct systems. The renal corpuscles and different segments of the renal tubules are irregularly distributed throughout the entire renal parenchyma surrounded by a connected tissue matrix, indicating the lack of zonation in the kidney of *L. chalumnae* (Fig. 1). Each nephron is composed of a renal corpuscle, a short neck segment, the proximal convoluted tubule that is divided into first and second segments, an intermediate segment and the distal tubule. The distal tubule of the nephron is connected to the collecting tubule [Figs 1 and 2(c)].

RENAL CORPUSCLE

The renal corpuscles occur singularly or in chains of two or three surrounded by connective tissue. They are variable in size measuring 85–200 μm (mean diameter is 116 μm) in paraffin and plastic transverse sections and oval or elongated in shape. The arterioles are not seen connected to the glomeruli, thus the vascular poles of the renal corpuscles are not identifiable. The renal corpuscles are not associated with any portion of the renal tubules [Fig. 2(a)–(c)]. The glomeruli are large and well vascularized with tufts of patent capillaries containing blood cells, with conspicuous mesangium. The visceral epithelium over the capillary loops is delicate consisting of a thin rim of cytoplasm with round nuclei bulging into the Bowman’s capsular space. The parietal epithelium of the Bowman’s capsule is composed of low cuboidal (5 μm tall) cells with round nuclei. The Bowman’s space is clear without any particulate matter [Fig. 2(b),(c)]. Abundant amounts of connective and haemopoietic tissues are present around the renal corpuscles [Fig. 2(b),(c)].

Fig. 1. Photomicrograph of a transverse section of the kidney of *Latimeria chalumnae* showing a renal corpuscle (RC) containing a glomerulus (G) and irregularly distributed renal tubule segment. CT, collecting tubule; DT, distal tubule; P1, first proximal tubule; P2, second proximal tubule. The directions dorsal (D), ventral (V), medial (M) and lateral (L) are indicated (2% glutaraldehyde–paraffin; 8 μm haematoxylin–eosin; ×200).
NECK SEGMENT

The short, straight and narrow neck segment measuring 50–55 μm in outside diameter connects the renal corpuscle with the proximal convoluted tubule. The parietal low cuboidal epithelium of the Bowman’s capsule becomes continuous with the non-ciliated larger cuboidal (15–20 μm tall) epithelium of the neck segment. The cell walls are indistinct but can be seen. The nuclei are round in shape. The cytoplasm is less abundant and lightly eosinophilic [Fig. 2(c),(d)].

Fig. 2. (a) Transverse section of the kidney of *Latimeria chalumnae* showing a chain of three renal corpuscles (RC). G, glomerulus (2% glutaraldehyde–paraffin; 6 μm haematoxylin–eosin; ×200). (b) Transverse section of a renal corpuscle containing a well-vascularized glomerulus (G). BS, Bowman’s space; C, capillary; CT, connective tissue; HT, haemopoietic tissue; M, mesangium; PE, parietal epithelium; VE, visceral epithelium of the Bowman’s capsule (2% glutaraldehyde–Epon; 1 μm toluidine blue; ×400). (c) Transverse section of a renal corpuscle (RC) and the neck segment (N). BS, Bowman’s space; C, capillary; CT, connective tissue; G, glomerulus; PE, parietal; VE, visceral epithelium of the Bowman’s capsule (2% glutaraldehyde–paraffin; 6 μm haematoxylin–eosin; ×400). (d) Longitudinal section of the neck segment. EP, epithelium; L, lumen (2% glutaraldehyde–paraffin; 8 μm haematoxylin–eosin; ×400).
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PROXIMAL CONVOLUTED TUBULE

The proximal convoluted tubule differs from the preceding neck segment in having a larger outside diameter and a taller epithelium. It is divided into two distinct regions referred to as the first proximal segment and the second proximal segment, respectively. The first proximal segment of the tubule begins abruptly where the neck segment ends. It is lined by columnar epithelium displaying a prominent brush border on its luminal surface. This segment is fairly regular in outline with a mean outside diameter of 118 μm in plastic sections. The height of its columnar epithelium is 35 μm and the cell walls are indistinct. The cells rest on a basal lamina and contain two spherical centrally placed nuclei with prominent nucleolus. The cytoplasm, which stains lightly especially in the apical region, contains vesicles and lysosome-like granules. The lumen, which contains abundant amorphous material, is usually large and 37 μm in diameter, but it is often constricted by the opposing prominent brush border of the surrounding cells [Fig. 3(a),(b)].

The second proximal segment of the tubule is nearly circular in transverse sections and has a somewhat smaller mean outside diameter (110 μm), but it differs markedly from the first segment in having a taller epithelium (45 μm), its lumen is relatively narrow measuring 29 μm in diameter and filled with amorphous material. The columnar cells have indistinct cell walls. They are surrounded by a basal lamina and contain two spherical nuclei, with prominent nucleolus, located in their central region. The cytoplasm is abundant, quite dense and contains vesicles and lysosome-like granules [Fig. 3(a),(c),(d)]. Both segments of the proximal convoluted tubule are surrounded by a connective tissue framework and display an equally prominent brush border on the luminal aspect [Fig. 3].

INTERMEDIATE SEGMENT

The intermediate segment is interposed between the second proximal segment and the distal tubule of the nephron and is circular or oval in outline in transverse sections. The mean outside diameter of the intermediate segment is the same as that of the preceding second proximal segment (110 μm) with diminished height of its columnar epithelium (25 μm) and its sharp lumen measuring 60 μm in diameter and appears empty [Fig. 4(a),(b)]. The cells rest on a basal lamina and are binucleate. The epithelium of the intermediate segment is devoid of cilia or brush border, confirming the observations of Millot & Anthony (1973a) [Fig. 4(a),(b)].

DISTAL TUBULE

The distal tubule segment of the nephron decreases in outside diameter measuring 80 μm in plastic sections, 55 μm in paraffin sections, as well as in the size of the lumen that is c. 45 μm in diameter in plastic sections, 30 μm in paraffin sections. It is not differentiated into early and late segments. Its epithelium is reduced in height to 17 μm in plastic and 15 μm in paraffin sections, and contains only one cell type; the intercalated cells are absent. The cells are cuboidal with distinct cell walls and devoid of brush border or cilia on the luminal surface. They are surrounded by a basal lamina and are binucleate. The cytoplasm stains lightly and uniformly [Figs 1 and 4(c)–(e)].
Fig. 3. (a) Transverse section of the first proximal segment (P₁) and second proximal segment (P₂) of *Latimeria chalumnae* kidney tubule. BB, brush border; BL, basal lamina; CT, connective tissue; EP, epithelium; L, lumen; LY, lysosome; N, nucleus; V, vesicle (2% glutaraldehyde–Epon; 1 µm toluidine blue; ×400). (b) Transverse section of the first proximal segment. BB, brush border; EP, epithelium; L, lumen; LY, lysosome in the epithelium (inset); N, nucleus (2% glutaraldehyde–Epon; 1 µm azure II; ×400). (c) Transverse section of the second proximal segment. BB, brush border. EP, epithelium; L, lumen; N, nucleus; V, vesicle (2% glutaraldehyde–Epon; 1 µm azure II; ×400). (d) A higher magnification view of a transverse section of the second proximal segment of the tubule. BB, brush border; BL, basal lamina; CT, connective tissue; EP, epithelium; L, lumen; LY, lysosome; N, nucleus (2% glutaraldehyde–Epon; 1 µm toluidine blue; ×800).

**COLLECTING TUBULE**

The transition between the distal tubule and collecting tubule is gradual, and its mean outside diameter (30 µm) and its epithelial height (8 µm) are markedly reduced from that of the preceding distal tubule segment. The cells are low cuboidal. They contain two small round nuclei and the cytoplasm stains lightly and contains vacuoles. Rod-like inclusions resembling bacteria are present in the cytoplasm. Intercalated
cells are absent in the collecting tubule epithelium [Figs 1 and 5(a),(b)]. The presence of renal corpuscles, proximal, distal and collecting tubules with large and small outside diameters suggests that they presumably represent different generations of nephrons. Abundant haemopoietic tissue is found interspersed between the renal tubular segments [Fig. 5(c)].

RENAL ARTERIES

Renal arteries measuring 130 μm in outside diameter are found in the substance of the kidney and arterioles originating from them, but they are not seen connected to the glomeruli and they have no association with the distal tubules [Fig. 5(d),(e)]. The arterial wall is composed of three concentric layers, i.e. tunica intima, tunica media and tunica adventitia containing collagenous tissue. The endothelium is separated from the tunica media by an internal elastic lamina. The nuclei of the endothelial cells bulge into the round lumen. The smooth muscle cells of the tunica media are not organized in distinct circular layers and contain granular material [Fig. 5(d),(e)].

COLLECTING DUCTS

The collecting duct system consists of small collecting ducts that join to form large collecting ducts, which in turn open into the mesonephric duct. The large collecting ducts have 130 μm outside diameter and 38 μm luminal diameter. They are lined by cuboidal to columnar epithelium surrounded by a prominent basal lamina, connective
tissue and smooth muscles. The epithelial cells are binucleate and contain lightly staining cytoplasm. The epithelium is devoid of intercalated cells [Fig. 6(a)].

MESONEPHRIC DUCT

The wall of the mesonephric duct is composed of an inner mucosal layer, a middle connective tissue layer and an outer smooth muscle layer. The luminal surface of the epithelium is characterized by an irregular contour and at places is thrown into folds. The epithelium varies from columnar to pseudostratified or stratified. The lumen of the mesonephric duct is irregular and appears empty [Fig. 6(b)].

DISCUSSION

The present study provides new information on the structure of the \textit{L. chalumnae} kidney, which may enhance the understanding of the osmoregulatory mechanism in \textit{L. chalumnae} and its unique position in vertebrate phylogeny.
The structure of the kidney of this juvenile female specimen is essentially similar to that of the adult specimens described by Millot & Anthony (1973a), but important differences and new structures are revealed by this study. These include relatively large renal corpuscles that are nearly half the size of those of the adult specimen and the presence of a non-ciliated neck segment, a second proximal segment structurally different from the first proximal segment and a distal segment in the renal tubules that have not been described before in \textit{L. chalumnae}.

Since the renal corpuscles and different tubule segments are irregularly distributed throughout the renal parenchyma, the kidney in \textit{L. chalumnae} lacks zonation unlike the kidney of elasmobranchs, archaic fishes \textit{Polypterus, Proopterus} (Lacy & Reale, 1985a; Hentschel & Elger, 1987, 1989; Ojeda et al., 2006) and amphibia (Uchiyama et al., 1990; Mobjerg et al., 1998, 2004). Moreover, the renal tubular segments and capillaries unlike those of the elasmobranchs (Lacy & Reale, 1985a, b) are not arranged in a counter-current system in the kidney of \textit{L. chalumnae}. 

\textbf{Fig. 6.} (a) Longitudinal section of \textit{Latimeria chalumnae} large collecting duct (CD) opening into the mesonephric duct (MD). BL, basal lamina; CT, connective tissue; EP, epithelium; L, lumen; N, nucleus; SM, smooth muscle (2% glutaraldehyde–Epon; 1 \(\mu\)m toluidine blue; \(\times 400\)). (b) Longitudinal section of the mesonephric duct showing a fold (F) of its epithelium (EP), connective tissue (CT) and smooth muscle (SM) layers of its wall. L, lumen (2% glutaraldehyde–paraffin; 6 \(\mu\)m haematoxylin/eosin; \(\times 400\)).
Several investigators have given measurements of the size of renal corpuscle in different fishes living in different environments. The cyclostomes possess the largest renal corpuscles (glomi) measuring 90–876 μm in length and 175–500 μm in diameter, followed by elasmobranchs (95–350 μm diameter), dipnoans (180 μm), freshwater teleosts (84–115 μm) and the marine teleosts, which have the smallest (48–100 μm) renal corpuscles (Marshall, 1934; Guyton, 1935; Grafflin, 1937; Hickman & Trump, 1969; Youson & McMillan, 1970a; Andrew & Hickman, 1974; Anderson & Loewen, 1975; Nishimura et al., 1983; Lacy & Reale, 1985a). In the crab-eating frog Rana cancrivora, which lives in a high-salinity environment, the renal corpuscles are 100–180 μm in diameter (Uchiyama et al., 1990). The present values of the size of the renal corpuscles of L. chalumnae (85–200 μm diameter) are closer to the values (180–280 μm) found by Lagios (1974), but much lower than the values (300–500 μm) reported by Millot & Anthony (1973a). Since the renal tissue used in this study was fixed in an iso-osmolar fixative thus avoiding shrinkage or swelling artefacts, the present measurements and those of Lagios (1974) of the diameter of the renal corpuscles of L. chalumnae are closer to their size in life. The size of the renal corpuscles of L. chalumnae is smaller than those of the elasmobranchs but is similar to those of the euryhaline freshwater teleosts and the crab-eating frog.

The structure of the Bowman’s capsule, glomerulus and renal tubule of L. chalumnae is essentially similar to those of cyclostomes, elasmobranchs, teleost, dipnoan fishes and higher vertebrates (Guyton, 1935; Hickman & Trump, 1969; Youson & McMillan, 1970a; Andrew & Hickman, 1974; Lacy & Reale, 1985a; Hentschel & Elger, 1989), suggesting that the basic structural plan of the nephrons was laid down very early in vertebrate evolution.

The highly vascular glomeruli seen in the kidney of L. chalumnae are apparently well adapted to eliminate excess water taken up by osmosis from the medium. According to Smith (1953), the glomerular kidney has evolved as a device to eliminate excess water from the body of early vertebrates found in the freshwater habitat. Although L. chalumnae is marine in its habitat, it does spend a lot of time in a mixture of sea and ground water. Until 1990, almost all specimens of L. chalumnae have been caught along the western coast of Grande Comoro in the western Indian Ocean. The volcanic Comoro Islands are reported to have a large number of underground caves into which large amounts of ground water seem to seep from the side of the island. During heavy rains, the sea water around the Comoro apparently becomes slightly brackish (Locket, 1980; Fricke & Hismann, 1990). Latimeria chalumnae is nearly iso-osmotic with normal sea water (Griffith & Pang, 1979). But it may become hyperosmotic to the brackish water it lives in, therefore facing an influx of water through the gill epithelium and oral mucous membrane as in marine elasmobranchs (Bone & Marshall, 1982).

The glomerular filtrate from the Bowman’s capsule apparently enters the short nonciliated neck segment and moves down the renal tubule. As in other fishes and higher vertebrates, monovalent sodium and chloride ions are presumably reabsorbed in the first proximal segment of the renal tubule, which is homologous to the mammalian proximal renal tubule (Hickman & Trump, 1969). The prominent brush border of the first proximal segment provides an extensive surface area for the absorption of electrolytes. The urine in L. chalumnae is iso-osmotic with the serum and contains higher concentrations of magnesium, phosphate and sulphate divalent ions than in the serum as in most marine fishes. The concentration of monovalent sodium and chloride
ions in the serum of *L. chalumnae* is very similar to marine teleosts (Griffith & Pang, 1979). Secretion of divalent ions most probably takes place across the tall epithelium of the second proximal segment of the renal tubule as in marine elasmobranchs and teleosts (Hickman & Trump, 1969; Evans, 1979; Bone & Marshall, 1982).

The non-ciliated intermediate segment in this juvenile specimen resembles that of the adult *L. chalumnae* nephron (Millot & Anthony, 1973a) but differs from the ciliated intermediate segment reported in archaic bonyfish *Polypterus* (Hentschel & Elger, 1989), dipnoi (Guyton, 1935; Ojeda, et al., 2006) and amphibia (Mobjerg et al., 1998, 2004). Since the neck segment is also devoid of cilia, it appears that the entire nephron epithelium in *L. chalumnae* is devoid of cilia, as is the case in birds and mammals (Hentschel & Elger, 1989). The distal tubule of the nephron of *L. chalumnae* consists of a single segment containing only one cell type (devoid of intercalated cells) in sharp contract to two segmented (early and late segments) containing two cell types: the principal cells and intercalated (mitochondria-rich) cells reported in *Polypterus*, *Protopterus* (Hentschel & Elger, 1987; Ojeda et al., 2006) and in several amphibians (Uchiyama et al., 1990; Mobjerg et al., 1998, 2004).

As the glomerular filtrate moves further down the renal tubule, presumably more monovalent ions are reabsorbed in the non-ciliated intermediate segment, the distal tubule and possibly the collecting tubule and duct in the kidney of *L. chalumnae*. The addition of intermediate and distal tubule segments to the renal tubule is thought to improve efficiency of the kidney in freshwater teleosts in conserving monovalent ions (Hickman & Trump, 1969; Evans, 1979). In support of this view, a physiological study has demonstrated that in the freshwater *O. mykiss* the distal tubule acts as a diluting segment in which sodium and chloride ions are reabsorbed from the tubule lumen (Nishimura et al., 1983).

Chemical analysis of the body fluid of *L. chalumnae* has shown that its serum contains urea, trimethylamine oxide (TMAO) and amino acids in concentrations similar to that of marine elasmobranchs, which are slightly hyperosmotic to sea water. The rectal gland as in elasmobranchs is apparently involved in sodium secretion. The total osmolarity of the serum of *L. chalumnae* is nearly iso-osmotic with sea water. Since the urea and TMAO levels in the bladder urine are identical to that of the serum, unlike elasmobranchs, the renal tubules in *L. chalumnae* are unable to reabsorb urea and TMAO from the glomerular filtrate (Griffith & Pang, 1979). In elasmobranchs, almost all of the urea is reabsorbed from the glomerular filtrate by the renal tubule segments that are arranged in a complex counter-current multiplier system (Hickman & Trump, 1969; Lacy & Reale, 1985a, b). In *L. chalumnae*, urea retention is presumably achieved by other mechanisms.

The presence of binucleate cells in the renal tubule segments and collecting ducts (except the neck segment) in this juvenile specimen as well as in the adult specimens (Millot & Anthony, 1973a) is a unique feature of the *L. chalumnae* nephrons among fishes. Giant binucleate cells have been reported in the renal tubules of toads (Mobjerg et al., 1998). These cells having twice the amount of DNA in their tetraploid nuclei may be able to easily replicate to replace damaged tubule cells when injured by toxic substances or microbial and parasitic infections as happens in mammalian liver (Dyson, 1978).

The renal arteries observed within the kidney of *L. chalumnae*, unlike teleost and mammalian arteries of similar diameter, which have tunica media consisting of circularly arranged smooth muscle cells (Andrew & Hickman, 1974; Gartner &
Hiatt, 2006), have a poorly developed tunica media consisting of irregularly arranged smooth muscle cells that contain granular material. Electron microscopy has shown that these modified smooth muscle cells called granular epitheloid or juxtaglomerular cells contain myofibrils, pinocytotic vesicles and numerous membrane-bound polyhedral secretion granules similar to those of teleost and mammalian juxtaglomerular cell granules, which contain renin, part of the renin–angiotensin system (Lagios, 1974). Although arterioles arising from renal arteries in the renal tissue are not seen connected to glomeruli in this study, Lagios (1974) has reported each glomerulus being supplied by a separate afferent arteriole arising from a single small artery in *L. chalumnae*.

Concerning the evolutionary relationship of the coelacanth to land vertebrates, the traditional view is that one group of sarcopterygians (lobe-finned fishes) represented by *L. chalumnae* is the closest living relative of the tetrapods. The chondrichthyes (cartilaginous fishes) and actinopterygians (ray-finned fishes including teleosts) are considered distant relatives of the tetrapods (Forey, 1988, 1990; Gorr & Klein-schmidt, 1993). Some investigators believe that the *L. chalumnae* is most closely related to chondrichthyes (sharks and rays). They list a number of features common to chondrichthyes such as osmoregulation through retention of urea and TMAO in the plasma and tissues fluids, a rectal gland, large eggs and the structure of the pituitary gland (Lovtrup, 1977; Lagios, 1982). Contrary to this view, Griffith & Pang (1979) have proposed that urea retention has evolved independently in elasmobranchs and *L. chalumnae*. This hypothesis is supported by the case of the crab-eating frog, which lives in estuaries and maintains serum osmolarity close to the medium with high concentration of urea; its retention is achieved primarily by the reduction in urine volume (Schmidt-Nielsen, 1979). It is apparent that this frog has invented urea retention independently to survive in a marine environment similar to elasmobranchs and *L. chalumnae*.

In conclusion, the structural features of the kidney of *L. chalumnae* revealed by this study, such as the large renal corpuscles with well-vascularized glomeruli, the neck segments, distinct first and second segments of the proximal tubules, the intermediate segments, the distal tubules and the collecting tubules of the nephrons, correlate well with their function in osmoregulation. The structure of its nephrons, lack of zonation of their components, in conjunction with some aspects of osmoregulation that *L. chalumnae* shares with teleosts, supports the view that *L. chalumnae* is closely related to euryhaline teleosts as the evolutionary cousin of tetrapods.

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**References**


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