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Non-respiratory blood vessels in *Latimeria* gill filaments

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A study of the blood pathways within the gills of *Latimeria* has been carried out using light and transmission electron microscopy. Clear evidence has been found for the presence of a secondary non-respiratory circulation in addition to the well-established respiratory pathway through the gill lamellae. All essential components of this system have been observed and have the same relationships and basic structure as comparable secondary systems in actinopterygian and elasmobranch fishes. These include a central venous sinus (CVS), arterio-venous anastomoses (AVAs) and central filament arteries (CFAs). AVAs connect both arterial vessels of the primary circulation and CFAs of the secondary circulation to the CVS. The latter contained many red blood cells.

The presence of this secondary circulation in *Latimeria* gills contrasts with the situation in the gills of the three living genera of lungfishes where a system possessing the essential features of the tetrapod lymphatic vessel system has been recognized. No suggestions of a true lymphatic vessel system were observed in *Latimeria*.

Other features of gill and vascular anatomy in *Latimeria* show its closer relationship to dipnoans than other groups of living fishes but evidence derived from this study of the secondary circulation clearly supports the view that the Dipnoi rather than *Latimeria* represent the living fishes most closely related to the tetrapods.

Keywords: *Latimeria chalumnae*; gills; blood vessels; arterio-venous anastomoses; central venous sinus; secondary vessel system

1. INTRODUCTION

Ever since the first extant coelacanth became known to the scientific world in December 1938, it has been recognized as one of the closest living relatives of tetrapods among the fishes. The only other competitors for this distinguished position in the phylogeny of vertebrates have always been the Dipnoi (lungfishes), some of which had even been classified as amphibians by their first investigators (Natterer 1837; Bischoff 1840; Krefft 1870).

Generations of zoologists, comparative morphologists and palaeontologists have favoured *Latimeria* over the dipnoans in this competition. Considerable interest in this question has been revived recently by the much disputed paper of Rosen *et al.* (1981). These authors seriously challenged the current view and proposed instead a sister group relationship between lungfish and tetrapods. Basically this idea coincides with much earlier notions vividly discussed before *Latimeria chalumnae* had been discovered. However, Rosen *et al.* were strongly contradicted by Jarvik (1981), Schultze (1981), Holmes (1985) and Panchen & Smithson (1987). In spite of various additional observations published by Forey (1980), Waehneldt *et al.* (1986), Fritzsch (1987), Chang (1991), Meyer & Dolven (1992), Hedges *et al.* (1993) and Meyer (1995), no unanimous agreement has been reached to date (Forey 1988, 1991; Gee 1990; Schultze 1994; Zardoya & Meyer 1996). These discussions have considered many different aspects of the problem but little attention has been given to the gill circulatory system. The discovery that the basic organization of the fish vascular system differs from that of tetrapods in one important respect, which has become apparent following detailed studies of the systemic circulation as well as the non-respiratory circulation in the gills (Vogel 1981; Vogel & Claviez 1981), may provide fresh evidence to illuminate the circumstances of the fish–amphibian transition.

The general fish-type circulation is characterized by a primary and a secondary system of blood vessels (primary vessel system (PVS) and secondary vessel system (SVS)). Both run largely in parallel and, although contrary to earlier notions, lymphatic vessels are completely absent. In spite of substantial variations, this basic scheme can be recognized in agnathans and elasmobranchs, as well as in actinopterygian fish (Favaro 1906; Vogel 1981, 1985*a*; for recent reviews, see Satchell 1991; Steffensen & Lomholt 1992; Ishimatsu *et al.* 1995; Olson 1996). There is, however, one group of fishes, the Dipnoi, in which the circulatory system does not conform to this general scheme. The circulatory system of lungfish is definitely of the type found in tetrapods: they have only the well-known single (i.e. not subdivided into PVS and SVS) system of blood vessels, but this is complemented by a distinct system of genuine lymphatics (Vogel & Mattheus 1998; Vogel *et al.* 1998; Morgan & Wright 1989). The essential character of the lymphatic vessel system is the absence

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of any inflow vessels from the blood circulation. It drains any surplus interstitial fluid and protein back to the venous part of the circulation.

In view of the ongoing discussions about the phylogenetic position of lungfishes versus *Latimeria chalumnae*, it is of considerable interest to explore whether *Latimeria* has a fish-type or, as in lungfish, a tetrapod-type circulation. Unfortunately, perfusion-fixed material for a detailed study of the coelacanths systemic circulation is not available. However, the gill vessel system can serve as a reliable representative of the general circulation.

The gill vessels of teleosts, elasmobranchs and dipnoans have been studied extensively over the last 25 years by various authors. For reviews of this work, see Hughes & Morgan (1973), Hughes (1979, 1980*a*), Laurent (1984), Randall (1985) and Olson (1996). Most attention has been given to the respiratory or lamellar circulation but, in addition, there is a non-respiratory blood pathway in fish gills which has eventually been recognized as part of the general SVS in fish (Vogel 1985*a*; Ishimatsu *et al*. 1995).

The main components of the non-respiratory (secondary) gill vessel system in 'typical' fish are:

1. The central venous sinus (CVS) (Vogel *et al*. 1973), which drains into branchial veins.
2. Arterio-venous anastomoses (AVAs), highly specialized in most species studied so far in detail (see Vogel *et al*. 1974, 1976; Dunel & Laurent 1980).
3. One or more central filament arteries (CFA), originating from an efferent filament or efferent branchial artery (Vogel & Kock 1981).

AVAs regularly connect central filament arteries and the CVS in fish gills. In addition, specialized AVAs connect efferent respiratory vessels of the gill filament to the CVS in a great number of species. In some teleosts such as the eel, and in elasmobranchs e.g. in *Raia clavata*, there are also corresponding AVAs in considerable number between afferent respiratory vessels and the CVS (Laurent 1984) and some even connect respiratory lamellae and the central sinus in the eel (Steen & Kruysse 1964).

Notwithstanding the fact that the functional role of the secondary circulation in fish is not well understood, neither in the gills nor elsewhere, the main micromorphological facts are well established, so that further comparative studies may be based on this knowledge.

Interestingly, in the gills of all three genera of lungfish, comparable non-respiratory blood pathways are lacking just as a secondary circulation is lacking in the rest of these fish (Vogel & Mattheus 1998; Vogel *et al*. 1998). Nutrient arteries do occur in the gill filament core of lungfish and so do small capillaries and veins, but specialized AVAs and a CVS are definitely absent (Gannon *et al*. 1983; Laurent 1984; Vogel & Mattheus 1998). Fortunately, specimens of well-preserved gill tissue material of *Latimeria chalumnae* were available to us to study the non-respiratory gill vessels of the coelacanth in some detail by light and electron microscopy.

For a general outline of the course of blood through the gills of the coelacanth, see the paper of Anthony & Robineau (1968). The anatomy of *Latimeria* gills, as well as ultrastructural details of its respiratory circulation in the gill lamellae, have been described by Hughes (1972, 1976, 1980*b*, 1995). The blood circulates within a meshwork of pillar cells which are a diagnostic feature of the lamellar circulation. The enclosure of connective tissue columns within the pillar cell cytoplasm is highly characteristic. Surprisingly, a similar enclosure of the supporting connective tissue network has also been found in mammalian lymph nodes (Hughes & Weibel 1972).

2. MATERIALS AND METHODS

For light microscopy, gill filaments were kindly provided by Professor G. von Wahlert, Staatliches Museum für Naturkunde Stuttgart, Germany, from *Latimeria* CCC no. 48 (Bruton & Coutouvidis 1991). Fixation of this specimen was by immersion in 4% formalin. Conservation had been in 80% isopropyl alcohol since 1966. Single gill filaments or small groups of filaments with the adhering gill septum have been embedded in a mixture of 90% butylmethacrylate and 10% methylmethacrylate. Seven series of sections, section thickness 1.5 µm over distances of up to 2 mm, provided the material for a light microscopic survey of the non-respiratory gill vessels. All sections were stained according to the method described by Richardson *et al.* (1960).

For transmission electron microscopy, gill filament tissue was taken from *Latimeria chalumnae* CCC no. 80 (Bruton & Coutouvidis 1991). This material consisted of grids prepared as part of an earlier study of the lamellar ultrastructure (Hughes 1980*b*) based on material fixed in glutaraldehyde at Iconi, Grande Comore in March 1972 as part of the British–French–American expedition. The composition of the fixative is given by Locket (1973).

3. RESULTS

(a) *Central venous sinus of gill filaments and septum*

Studies on serial sections of *Latimeria* gill filaments by light microscopy revealed the existence of large venous intrafilamentar blood spaces continuous with one another and all containing many red blood cells (see figures 1, 2, 7 and 8).

Similar venous blood spaces are found in the gill septum and there are many connections between the filamentar and septal venous blood spaces.

The wall of these venous sinusoidal vessels within the gill filaments (and the septum) is extremely thin, measuring between 0.1 and 0.5 µm outside the regions where cell nuclei bulge into the vessel lumen. Electron micrographs prove that the vessel wall is made up virtually of only a thin endothelial cell layer (see figures 3 and 4). Interstitial extensions of these cells are fairly regularly observed. Often these endothelial extensions establish contact with extensions of other interstitial cells. Filaments within the interstitium, however, seldom insert on these extensions. Contact between neighbouring endothelial cells is usually made by elaborate interdigitations (see figure 4). Occasionally one can see parts of a basement lamina, but more often it is completely absent. Red blood cells are commonly observed within the lumen of these vessels (see figure 3).

(b) *Gill filament arteries*

The wall of the afferent and efferent filament arteries is made up of three different cell layers (see figure 5). The intima is purely endothelial. There is a rather broad basement membrane which displays a web of fibres in our

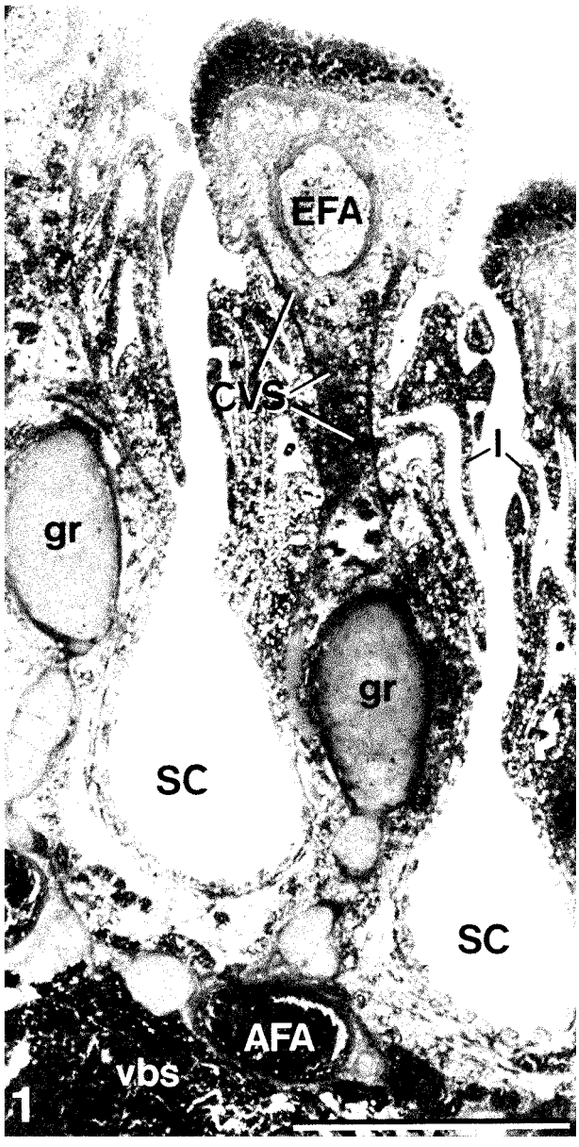

Figure 1. Light micrograph of transverse section of gill filaments, showing attachment to interfilamentar septum. Symbols: AFA, afferent filament artery; EFA, efferent filament artery; vbs, venous blood space in interfilamentar septum; sc, septal channel; l, lamella; gr, gill ray; CVS, central venous sinus. Bar represents 1 mm.

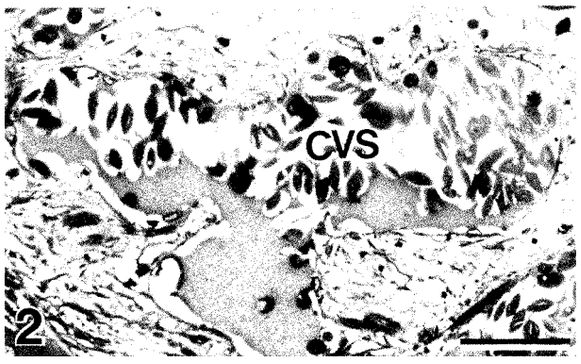

Figure 2. Higher magnification of central region of a filament to show part of central venous sinus (CVS) containing many red blood cells. Bar represents 50 μm.

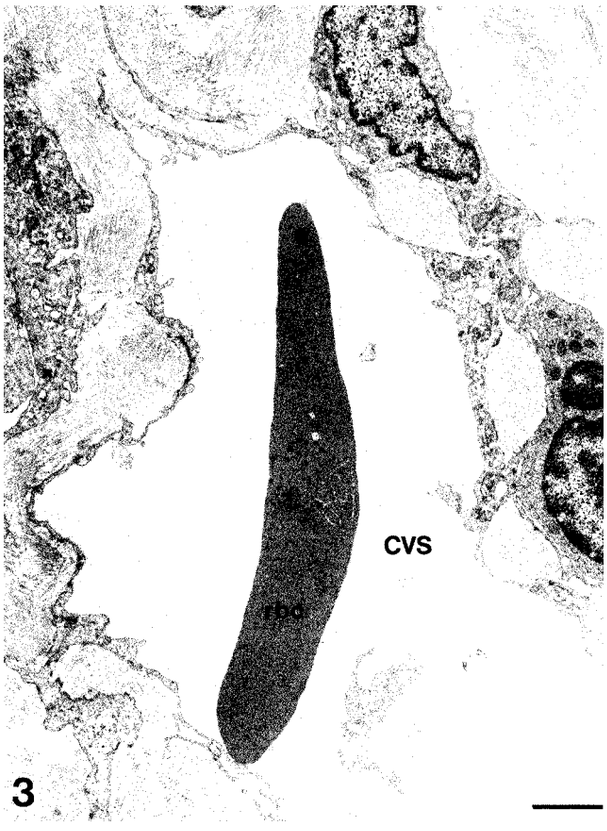

Figure 3. Electron micrograph of section through central venous sinus (CVS) which contains a single red blood cell (rbc). Extensions of endothelial cells reach out into interstitial space. Note typical irregular lining of the CVS. Bar represents 2 μm.

electron micrographs (see figure 6). The media of these arteries contains about three layers of smooth muscle cells within sheets of collagenous connective tissue fibres. These continue into the poorly defined adventitia. In contrast, the wall of arterial vessels within the filament core consists of only a single layer of smooth muscle cells separated from their endothelial cells by a thin basement membrane. Central filament arteries take their origin repeatedly throughout the filament from small branches of the efferent filament artery and occasionally also by very small arterio-arterial anastomoses.

(c) *Arterio-venous anastomoses*

Central filament arteries are connected at numerous points along the filament by specialized AVAs to the venous sinusoids. In the distal part of three gill filaments we found an average of 6.3 of these anastomoses per millimetre of filament length. Similar anastomoses (AVAeff) fairly regularly connect efferent lamellar arterioles to the CVS (see figures 7 and 8). The average number of AVAeff was 5.3 per millimetre of filament length in the same three gill filaments. These AVAeff are highly specialized vessels by light microscopic as well as by ultrastructural criteria (see figures 7–9): (i) they contain conspicuously large endothelial cells; (ii) the endothelial cell layer is surrounded by one or two layers of specialized cells which

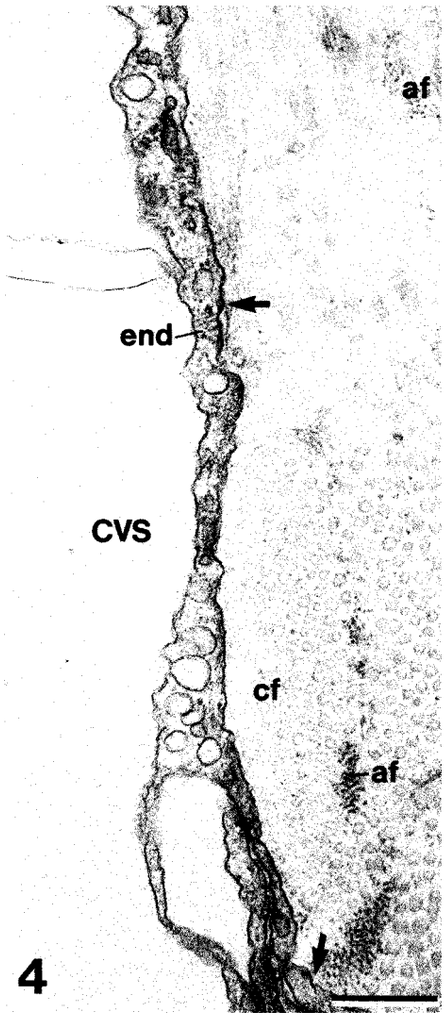

Figure 4. Section through endothelial lining (end) of central venous sinus (CVS). Note absence of basement membrane. Other symbols: af, anchoring filament attached to abluminal membrane of endothelial cell (arrows); cf, collagen fibres. Bar represents 0.5 µm.

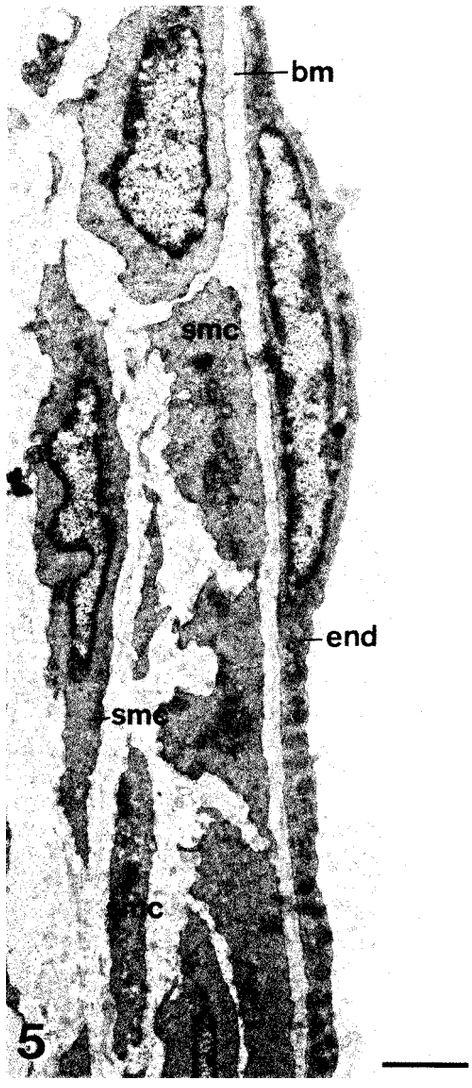

Figure 5. Electron micrograph of longitudinal section through efferent filament artery. Endothelial lining (end) with flattened nucleus has well defined basement membrane (bm). Two to three layers of smooth muscle cells (smc) form the media. Bar represents 2 µm.

would be classified as smooth muscle cells by light microscopic criteria; and (iii) the lumen of these AVAs is always relatively narrow, both with respect to wall structure and when compared with other types of blood vessels in *Latimeria* gills (see figures 7 and 8). The endothelial cells seem to be particularly voluminous and elongated at the arterial beginning of an AVA. Quite often they protrude into the arterial vessel from which the AVA originates. At the venous end of these AVAs, endothelial cells are usually smaller than those at the arterial beginning and also more spherical. They protrude in clusters around the AVA opening into the CVS (see figure 7).

In most cases, we have found the AVAs tightly closed (see figures 7 and 8). However, occasionally one or two blood cells appear trapped within an AVA. The circular layers of cells outside the endothelial layer are most probably smooth muscle cells (see figures 8, 9 and 11). These cells show no unusual features using light microscopy. AVAs very often divide into two branches in *Latimeria* gill filaments, thus reaching different parts of the CVS. We have also seen twin AVAs running largely in parallel. If AVAs run over a substantial distance they always take a rather tortuous course.

In electron micrographs, AVA endothelial cells show an electron dense cytoplasm. These cells are not only much more voluminous than ordinary endothelial cells in other blood vessels of *Latimeria*, they also differ in a number of other characteristic features. Although Weibel–Palade bodies are fairly regularly encountered in arterial endothelial cells and in marginal channels of the lamellar blood pathway, they almost never occur in AVA endothelial cells. In the extremely thin CVS endothelia they also seem to be scarce. AVA endothelial cells usually show a much better developed set of cell organelles than endothelial cells of other blood vessels. Notably the Golgi apparatus is prominent in some AVA endothelia and multivesicular bodies are repeatedly encountered (see figure 10). Mitochondria are particularly numerous. The cisternae of both smooth and rough endoplasmic reticulum are more clearly delineated than in

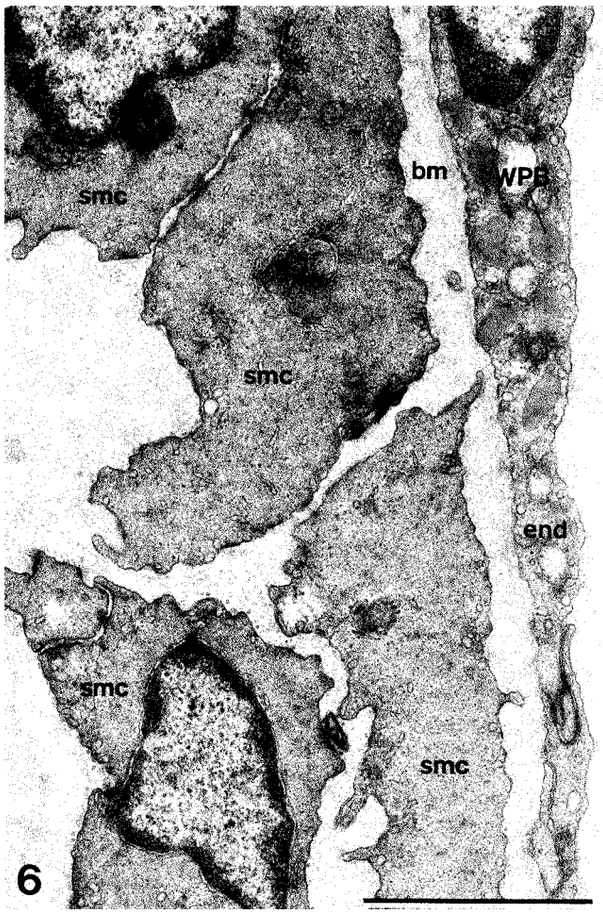

Figure 6. Wall of efferent filament artery. Weibel–Palade bodies (WPB) in endothelial cells and coarse fibres of basement membrane (bm). Other symbols: smc, smooth muscle cell; end, endothelial cell. Bar represents 2 µm.

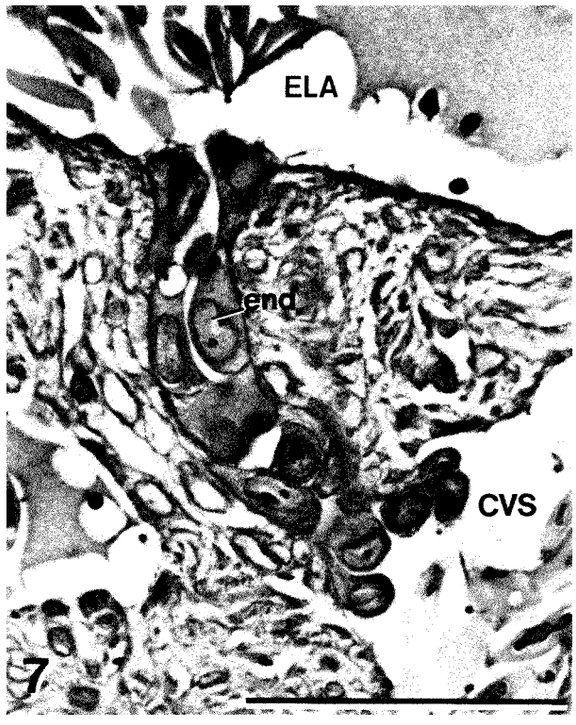

Figure 7. Light micrograph of longitudinal section through an AVA connecting efferent lamellar artery (ELA) and central venous sinus (CVS). Other symbols: end, endothelial cell. Bar represents 50 µm.

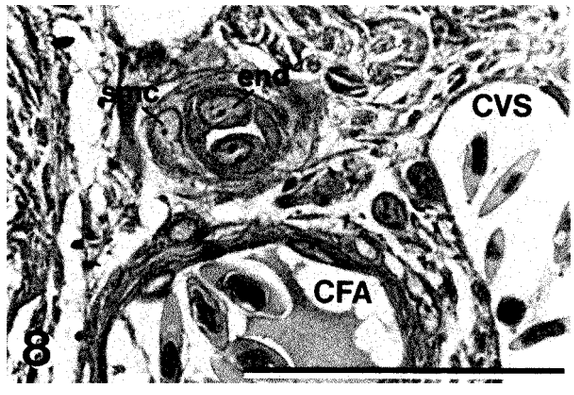

Figure 8. Light micrograph of transverse section through an AVA showing large endothelial cells (end) which almost fill the lumen of the anastomosis. Well-developed sheath of smooth muscle cells (smc) can be seen. Other symbols: CVS, central venous sinus; CFA, central filament artery. Bar represents 50 µm.

normal endothelial cells. Furthermore, small and large caveolae and vesicles are abundant in AVA endothelia. The most characteristic feature of these cells, however, is the presence of long, tentacular extensions of the cell surface, which may reach far into the AVA lumen (see figure 11). The cell bodies of AVA endothelia often bulge into the lumen of the AVA, narrowing it to a slit or even occluding it completely. AVA endothelia of *Latimeria* often interdigitate at their basal part. Here they are connected to each other by local point-to-point connections and by simple tight junctions. Desmosomes, however, have not been found between these cells nor between any other endothelial cells in the gill vessels. A very faint basal lamina delimits the abluminal cell membrane of the AVA endothelial cells, but it is completely lacking at the multiple myoendothelial contacts (see figure 11).

AVA cells outside the endothelial cell layer have a basal lamina of their own (see figure 11). Their mitochondria are located in the centre of the elongated cell body. Contractile filaments are scarce. In some of these cells they seem to be almost completely absent. Nevertheless we would classify these cells as (specialized) smooth muscle cells. Close myoendothelial contact is evident at many points in AVA cross sections. Towards the venous end of the AVAs the circular layer of smooth muscle cells gets thinner and thinner. No reliable indications for an innervation of the AVAs have been observed. Only once a possible synaptic swelling of a nerve fibre, containing small round and oval clear vesicles as well as a few dense cored vesicles, was found next to an AVA smooth muscle cell in *Latimeria*.

4. DISCUSSION

(a) *Secondary circulation of* Latimeria *gills*

Venous blood spaces in the gill filaments of the coelacanth *Latimeria chalumnae* clearly constitute a CVS as it is

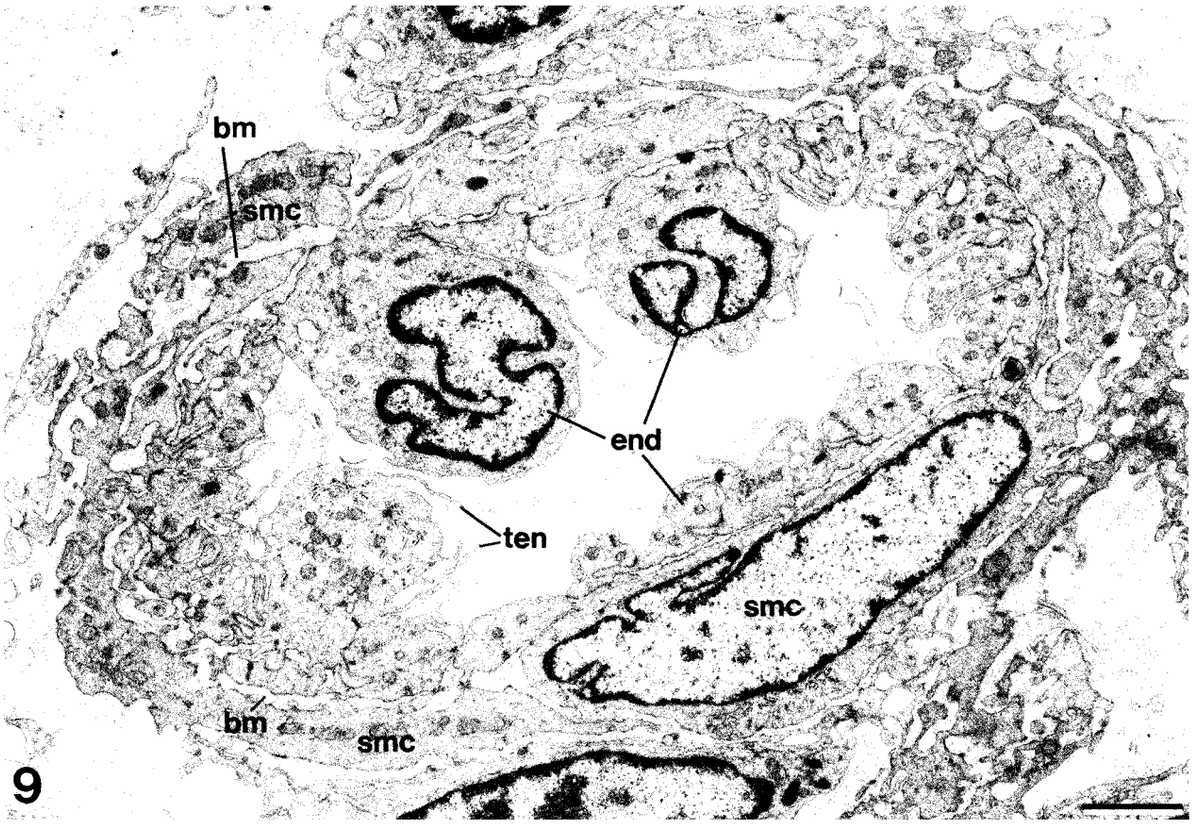

Figure 9. Electron micrograph of AVA (transverse section). Two prominent nucleated endothelial (end) cell bodies bulging into the lumen are visible together with basement membrane (bm). The nucleus of one surrounding smooth muscle cell (smc) is also visible and the presence of at least one other smooth muscle cell can be discerned. Other symbols: ten, tentacular extension of endothelial cell. Bar represents 2 µm.

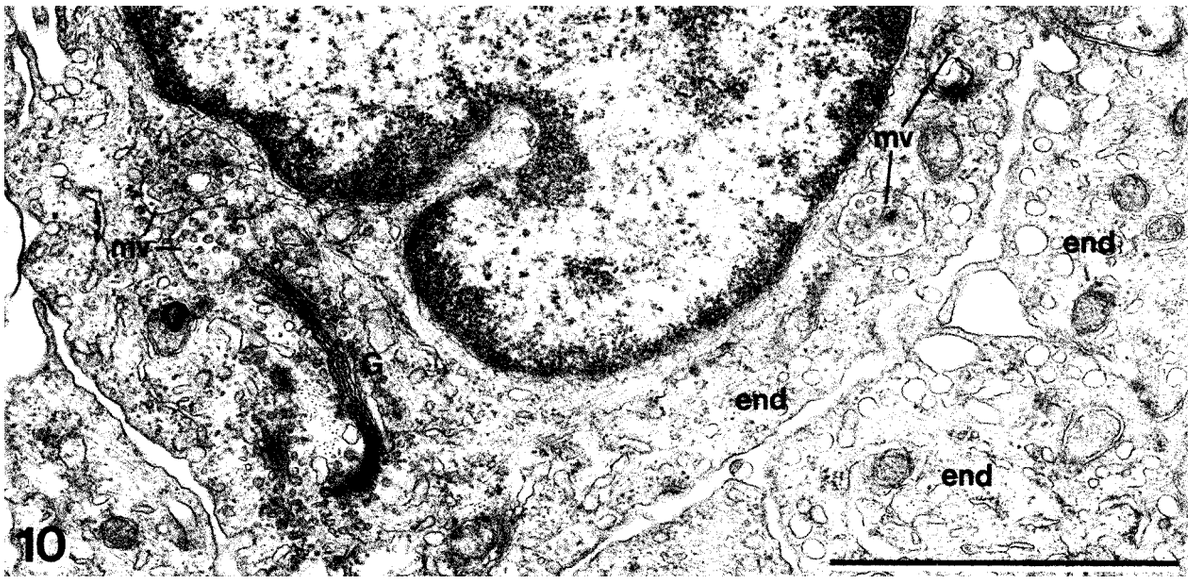

Figure 10. Endothelial cells (end) of AVA. Well-developed Golgi apparatus (G) and multivesicular bodies (mv) are clearly visible. Bar represents 2 µm.

found in gill filaments of actinopterygian and elasmobranch fishes. The term 'sinus' indicates that this vessel may be extremely variable in shape as well as in diameter. Usually it is divided into several compartments, apparently more or less independent of each other. In some fish the CVS has a more regular appearance as has been observed by Olson (1996) in trout gills as well as in some other actinopterygian species. However, its most essential feature is the continuity between different components and, as these vary between species with no apparent functional

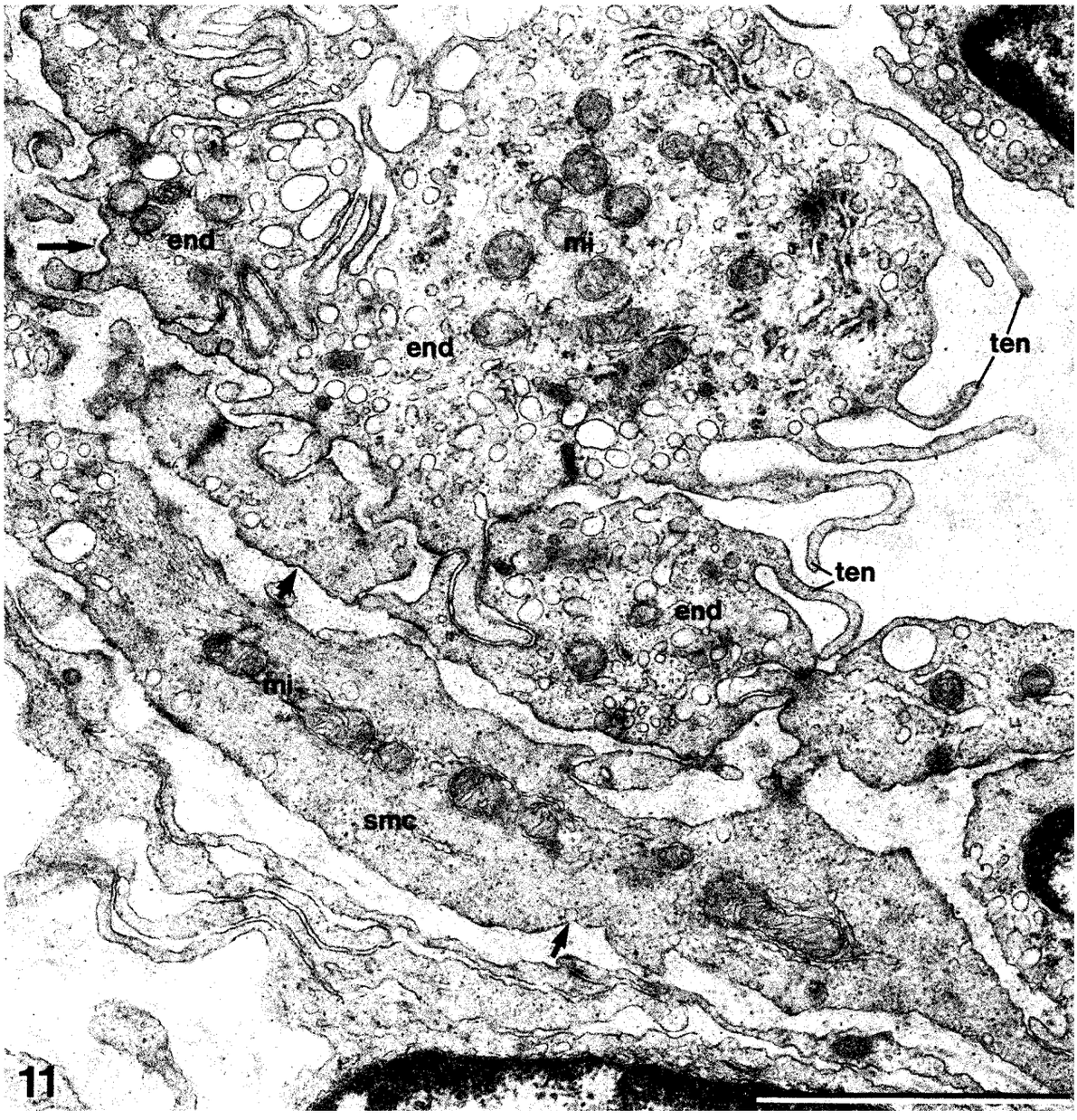

Figure 11. High magnification electron micrograph of endothelial cells (end) shown in figure 9. Tentacle-like extensions (ten) of luminar cell surface are characteristic of this type of endothelial cells. Other symbols: smc, smooth muscle cell; mi, mitochondria; arrows, basement lamina. Bar represents 2 µm.

correlates, it would seem inadvisable at the present time to distinguish any of the components with separate terms.

A well-known character of the CVS is its extremely thin wall, virtually a single thin endothelial cell layer on a discontinuous basal lamina only. In this respect the different parts of the CVS in *Latimeria* gills closely resemble lymphatic vessels, as does the CVS in other fish (Olson 1996). In fact many distinguished scientists were mislead by such similarities and had regarded the CVS as part of a lymphatic vessel system in fish (Vogel *et al.* 1973).

In addition, endothelial cells in the wall of the intrafilamentar sinuses of *Latimeria* occasionally have fine interstitial extensions, which is another feature they have in common with lymphatic endothelial cells. This can only be seen by electron microscopy (see figure 3). However, these endothelial extensions in *Latimeria* usually contact other cells in the interstitial space, whereas extensions of lymphatic endothelia (in lungfish and tetrapods) exclusively contact interstitial ('anchoring') filaments. There can be no doubt that 'anchoring' filaments are also present around the CVS vessels and that they are affixed to the outer endothelial cell membrane much in the same way as they are on lymphatic endothelial cells of tetrapods (see figure 4). In addition, cell contact between CVS endothelial cells is often established by complicated interdigitations, another feature commonly found in lymphatics. However, red blood cells are often found in the coelacanth's intrafilamentar venous sinuses (see figures 2 and 3). As our gill tissue

for light and for electron microscopy is from two different specimens, this is most probably not a chance observation. Moreover, it parallels observations on the CVS in gills of many actinopterygian fish.

A most important aspect, however, is the finding that the CVS in *Latimeria* gills is connected to both non-respiratory and respiratory arterial vessels by specialized AVAs much in the same way as it is in the gills of actinopterygian and elasmobranch fish. Indeed the presence of these connections rules out the possibility that the CVS could be a lymphatic vessel (Vogel *et al.* 1973). In addition, the presence of red blood cells within the CVS proves that at least some inflow of blood into the CVS must be possible. In other fish species, AVAs have occasionally been found to be far more open. So the fact that AVAs in fish gills are usually closed when viewed in sections for light or electron microscopy might only represent the situation shortly before fixation. It could also be an unavoidable effect of the fixation itself. Hughes *et al.* (1982) have studied the reactivity of AVAs in live fish and in perfused gill preparations. Perfusion of non-respiratory gill vessels in the cod, *Gadus morhua*, has also been studied recently under hypoxic conditions (Sundin 1995), and in rainbow trout, *Oncorhynchus mykiss*, after the injection of adenosine (Sundin & Nilsson 1996). The amount of blood or plasma that flows through the CVS has been shown to be a substantial part of the cardiac output in some teleosts under experimental conditions (Hughes *et al.* 1982; Randall 1985; Sundin & Nilsson 1992).

It appears reasonable to suggest that opening and closure of AVAs in fish gills is well regulated according to the particular physiological requirements of the fish. The ultrastructural observations on AVA endothelial cells in *Latimeria* gills, especially the many cytoplasmic cell organelles, strongly suggest a high degree of activity. Possibly these cells are concerned with active uptake or release of fluid from or into the AVA lumen. Consequently they might change their cell volume quickly and so regulate the luminal diameter of the AVA and hence the inflow of blood into the CVS. Smooth muscle cells of the AVAs, having only few contractile fibres, may have a more static role in support of such dynamic processes within the endothelial cells. There is no well-established evidence for innervation of AVAs (Laurent 1984). Humoral agents may be the important regulators of the AVAs in *Latimeria* just as in trout and cod (Nilsson 1984).

Unfortunately it is still not clear what functional role the CVS in fish gills may serve. An osmoregulatory task has been suggested by a number of authors (e.g. Vogel *et al.* 1974; Laurent 1984; Olson 1996). But for *Latimeria*, with its blood plasma being almost iso-osmotic with surrounding seawater (Griffith 1980), osmoregulation may be a minor problem which could be solved mainly by the kidneys as chloride cells appear to be absent in the gills (Hughes 1980*b*, 1995).

Independently, Nilsson (1984) and Vogel (1985*b*) have suggested the hypothesis that, in addition to a role in osmoregulation, the intrafilamentar (non-respiratory) blood pathway in fish gills might also serve as a way to provide an intraventricular oxygen supply to the inner spongy (trabecular) part of the heart muscle in critical hypoxic situations. Thus it could be envisaged as an emergency oxygen reserve system mainly for the heart, complementing the coronary supply. Coronary vessels, where present at all, mainly serve the outer, compact part of the fish heart. Recent results of physiological studies (Sundin 1995; Sundin & Nilsson 1996) certainly do not contradict this idea.

Extending such speculation to the secondary vessel system of fish as a whole may help interpret some features of the skin vasculature. In those fish that have been studied in detail it has been shown that the skin outside the scales is served exclusively by secondary vessels (Vogel 1985*a*). In this context it is of interest to recall that under unfavourable conditions fish may develop a pinkish or reddish 'flush' of their skin. This may be due to the opening of anastomoses, which allows the entry of red blood cells to this superficial part of the circulation and thus perhaps can supplement the oxygen supply to the heart (and the body as well).

There are also occasional AVAs between afferent lamellar arterioles and the CVS (AVAaff) in gills of *Latimeria*. However, these are extremely uncommon. They were observed only twice and by chance in single sections. A systematic search through serial sections gave a negative result. This compares well with the situation in teleostean gills where some species have almost no AVAaff whereas they are abundant in others.

In conclusion, all main components of a non-respiratory (secondary) gill vessel system are present in the gills of *Latimeria*. Much to our surprise they show many similarities to those of actinopterygian fish, which previously have been studied in detail with the electron microscope (Vogel *et al.* 1974, 1976; Dunel & Laurent 1980).

(b) *Phylogeny and* Latimeria *vascular system*

From a phylogenetic point of view the most important result of this study lies in the fact that *Latimeria* is closely linked to all other fish (except lungfish) in possessing a fish-type pattern of gill vascular organization (table 1). The difference from lungfish in this respect is fundamental. All three genera of extant lungfish have a vessel system in their gill filament core which follows the basic tetrapod-type scheme: no CVS, no specialized AVAs, but lymph capillaries plus microlymph hearts or 'cisternae' (Morgan & Wright 1989; Vogel & Mattheus 1998; Vogel *et al.* 1998) (see tables 1 and 2). If the results on the gill vessel system of *Latimeria* were extrapolated to the systemic circulation it would be expected that the coelacanth has a general secondary vessel system as other fish having a non-respiratory (secondary) gill circulation. Lungfish systemic vasculature, however, is of the tetrapod-type, just as is their gill circulation (Vogel & Mattheus 1998; Vogel *et al.* 1998). Both tetrapods and dipnoans have a blood vessel system that is not subdivided into a primary and secondary circulation and both have in common a distinct lymphatic vessel system which is completely absent in typical fish. The main common features of the lymphatic vessel system in Dipnoi and tetrapods, as well as the few minor differences, are summarized in table 2. On this basis it appears reasonable to conclude that, of the living groups of fishes, Dipnoi are more closely related to tetrapods than are the coelacanths. This viewpoint would support scheme 2 of figure 12.

In some other features of its vascular system (e.g. simple heart), *Latimeria* is also close to other fish, but the

Table 1. *Comparison of secondary circulation in a 'typical fish' with that in* Latimeria *and the situation in Dipnoi*

	fish	*Latimeria*	Dipnoi
gill circulation			
primary vessel system	present	present	present
arterio-venous anastomoses eff.	present	present	absent
arterio-venous anastomoses aff.	present	present	absent
central filament arteries	present	present	not comparable
central venous sinus	present	present	absent
ultrastructure of CVS and AVAs	very similar		not applicable
peripheral systemic circulation			
primary vessel system	present	present	present
inferior vena cava	absent	present	present
inter-arterial anastomoses	present	not investigated	absent
secondary vessel system	present	not investigated	absent

Table 2. *Comparison of main features of the lymphatic systems of mammals, lower tetrapods and dipnoan fish*

	mammals	lower tetrapods	Dipnoi
peripheral network of lymphatic capillaries	present	present	present
ultrastructure of lymphatic capillaries		essentially the same	
peripheral contractile units	contractile vascular segments (valved)	lymph hearts (valved)	micro-lymph hearts or 'cisternae' (valved)
collecting lymphatics	present	usually present	absent
connection to systemic veins	single, close to heart	many, into large veins	very many, peripheral, into blood capillaries

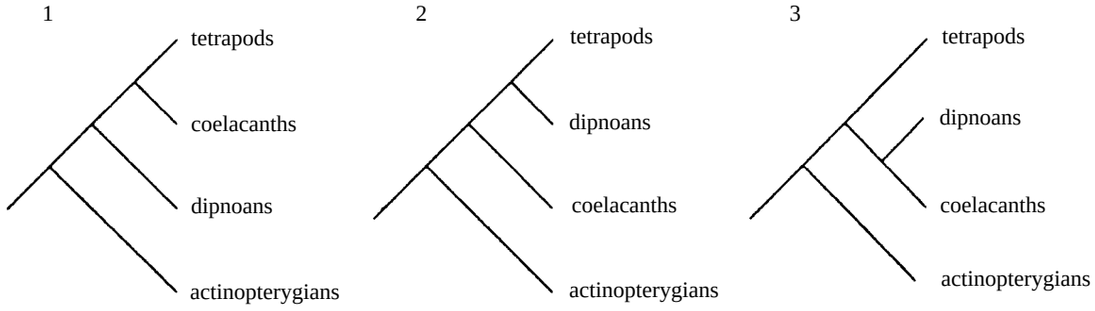

Figure 12. Diagram to illustrate three possible relationships between tetrapods and the main groups of living bony fishes. The more 'classical' scheme is shown in scheme 1 but the main findings of this paper support scheme 2.

presence of an inferior vena cava associates it with dipnoans. These fish depend on a lung for air breathing to a variable extent, but in *Latimeria* the swimbladder is full of lipid and has no respiratory function. The air-breathing habit of dipnoans is associated with a partitioning of oxygenated and deoxygenated blood flows in the heart, which has a more complex organization of its chambers. An important feature present in dipnoans, but not other air-breathing fish, is the return of oxygenated blood from the lung directly to the left auricle. The aortic arches are also significantly modified so that it is possible for blood to flow directly from the ventral to the dorsal aorta. Such features, which may be regarded as preadaptive to a dependency on lung breathing, are absent from *Latimeria*.

Several gross anatomical features have been used in discussions of phylogenetic relationships, including the extent of the interbranchial septa (Sewertzoff 1924). Among living fishes this is most developed in elasmobranchs where its extensions serve as flap valves covering the external gill slits and there is no operculum. In teleosts the septum is very much reduced and so reduces the resistance to water flow between the lamellae and is correlated with the well-developed operculum. Among primitive actinopterygians such as *Amia*, the septum is well developed proximally but the gill filaments are free for about one-third of their length. The condition in the dipnoan, *Neoceratodus*, is also intermediate. In this case the interbranchial septum is extended into the region where the filament tips have separated. The presence of these

extensions is more evident in *Latimeria*, where the main septum splits proximally but continues and maintains contact between filaments of individual hemibranchs except at their tips. These observations suggest a separation into three major types of septal organization: elasmobranchs, Actinopterygii including teleosts, and *Neoceratodus*, being close to the condition in *Latimeria* (Hughes 1972, 1980*b*).

It is concluded that the study of several different aspects of *Latimeria* gill morphology confirms the close relationship among living fishes between *Latimeria* and Dipnoi. However, what is known of the vascular organization of *Latimeria* strongly suggests its close connection to other fish, whereas the circulatory system of the Dipnoi much more closely resembles the condition in tetrapods.

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